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EXPERIMENTAL RICKETS

BY

EDWARD MELLANBY, M.A., M.D.



LONDON

PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE

1921

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Medical Research Council.

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EXPERIMENTAL RICKETS

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¹ The spelling 'vitamin' has come into general use since this Report was set in type.

I. INTRODUCTION

THE experimental work and results to be described in this paper are the outcome of an investigation into the cause of rickets extending over the last five years. The work is being actively prosecuted and the publication is to be regarded as of an interim nature. Two earlier accounts (1) have already been published both representing lectures given at institutions. In neither case was it possible to present more than a small amount of evidence in support of the deductions made, and it seemed desirable that a publication which supplies more evidence and fuller experimental details of the work performed should be forthcoming. It is not thought that any part of the research is complete but it has reached a stage when several points of practical importance as regards rickets are established and evidence of these facts is now offered.

As the work has proceeded, it has necessitated the introduction of more accurate and refined methods, which have made many of the earlier experiments appear crude. Points which were considered as of little account now appear of greater importance and vice versa. In spite, however, of the crudeness of the earlier work, I shall describe some of it; for it is the refining process that results from prolonged research of this type which illustrates more vividly the relative importance of the facts and adds to the interest by revealing the difficulties encountered.

The investigation was undertaken in the first place to find the actual cause or causes of rickets and the work was made as comprehensive as possible. When I was satisfied that some of the most important of these were established, it was then necessary to examine each factor more closely and especially in relation to the other causative agents. The closer examination carried out under precise conditions obviously resulted in a more balanced view as to the relative importance of the facts, as well as leading to more correct interpretations of the experimental results.

All dietetic problems must ultimately submit to quantitative experiment. During the earlier experimental period only qualitative work was possible. Any one with experience of feeding experiments must be aware of the difficulty of getting most animals to eat measured amounts of food when the diets are not physiologically complete. The fact that such diets tend to produce ill health militates against complete success along these lines, and it is only after much experience that it is possible to obtain dietetic mixtures which, while producing results quickly, are eaten completely and with relish.

A criticism that can be made against work of this nature is that, to ensure success, it is essential that the experiments should be made on animals of the same type and breed and that, when absolute comparison is wanted, only puppies of the same litter should be used. To those who think that heredity plays an important part in

the aetiology of rickets, this criticism will particularly appeal. My attitude, as I imagine is that of most physiologists, is to place the hereditary factor as the cause of a particular disease in the last category for investigation and to bring it into prominence only when all other explanations fail. It is quite possible that heredity may be of some importance in rickets, but it has not up to the present materially interfered with the results of these experiments so far as I have been able to observe.

It is true that I have attempted to work on litters of puppies so far as was possible but results will be given and deductions made, more particularly in the earlier experiments, when no such condition holds. Work of this nature is much simpler when each animal is comparable with another animal but, on the other hand, experiments on dissimilar animals certainly lead to results which might be missed working under the more ideal conditions. Such factors, for instance, as size and weight, rate of growth and general habits of the various types of puppy, all of which, as will be seen, are of great importance in the study of rickets, stand out prominently before the investigator when, as has happened in some of the earlier experiments, any puppy which could be procured was investigated. There is also as great a difference in the weight, rate of growth, and general habits of individual children as there is in puppies, and the disease in children is the ultimate problem awaiting solution.

The work to be described has been done on a very large scale involving the dieting of nearly 400 puppies, and it will be seen to include the whole problem of nutrition. A moment's consideration would lead one acquainted with the science of dietetics to foretell the unlimited nature of a problem of this type. It is true that recent investigations of deficiency diseases such as beri-beri and scurvy have been circumscribed and yet apparently so satisfactory that it would appear that a nutritional disease may be very limited in its aetiology; but this does not hold in rickets. In fact there is a temptation to suggest that knowledge of both beri-beri and scurvy would be greatly increased if the experimental methods were extended to include the consideration of other elements of diet and mode of life, as well as the respective vitamins involved in the diseases. This has been done to some extent by Braddon and Cooper (5) and Funk (6), and, as the result, there is evidence that carbohydrate plays a part in the development of avian polyneuritis. It may happen that, in the case of beri-beri and scurvy, as in this rickets investigation, the limited hypothesis as to their aetiology, in which only vitamins are taken into account, will have to be extended so as to include not only other elements of nutrition but also the general metabolism.

One point, which has come most prominently before my mind as the work has progressed, is the unity of a complete diet and the interdependence of the dietetic elements. This might have been expected. Knowledge has been accumulating in recent years which emphasises the importance of balanced diets, and we know that to cut out one element of the diet means not only the absence of that element, but also the ineffective action of other elements. For instance, when carbohydrates are removed from the diet, fat is

ineffectively oxidized, and there is also good evidence that in their absence animals and plants are incapable of synthesizing proteins from the amino acids. This is a simple instance of the absence of one element upsetting the action of other elements. When carbohydrates are given in large excess we know from the investigations of Bloch (33) on the diets of children in Denmark during the war the dire results that may ensue. When protein is cut out of the diet, although the direct and immediate effects of its absence are not properly understood, yet both man and animal are incapable of eating the diet for more than a few days. Not only does this inter-relationship hold between different elements of the diet, but also between these elements and the general metabolism of the body. For instance, however much fat there may be in the diet of an animal living in extreme cold, great discomfort is experienced under these conditions unless there is also a large amount of protein. Whereas fat is desirable and eaten with satisfaction where cold and exposure are to be endured, it is generally nauseous to those confined or living in a hot or sultry climate.

These few instances cannot fail to impress the investigator of dietetic diseases that any problem, which at first sight appears limited, may ultimately have to include a consideration of other factors in addition to the one that at first appeared all important. It has so happened in this investigation.

While it is evident that a vitamine, probably Fat-soluble A, occupies a position of prime importance in the aetiology of rickets, it is undoubted that this vitamine works in a close relationship with the other dietetic elements, and, moreover, with the general activity of the body, so that all the other factors both of diet and environment must be analysed in relation to it.

There is one other fundamental difficulty in investigations of this type, in that the facts observed are expected to explain the production of a disease in a type of animal different from that upon which the experiments are made. This difficulty holds in all animal experimental work and the danger of arguing from one animal to another cannot be emphasized too strongly, more especially in dealing with questions of diet. If, for instance, the work on scurvy had been attempted on rats instead of guinea-pigs, no progress could have been made, for these animals do not develop the disease. That is to say the human being, guinea-pig, and rat react differently to the absence of the anti-scorbutic factor. Again it will be seen that while the resemblance between the anti-rachitic factor and Fat-soluble A is so similar as to warrant the suggestion that they are the same things, yet the evidence is not complete. The apparent difference may, of course, be due to the difference of the metabolism of the rat upon which the experiments involving the distribution of Fat-soluble A have been made, and the puppy which has been the experimental animal in this work on rickets. It is not likely that the difference between a puppy and an infant is as great as that between a rat or guinea-pig and an infant, yet caution is necessary in applying the results of puppy experiments to the problem of rickets in children.

In addition to the solution of the practical problem of rickets, one other object has been constantly before my mind since it.

became evident that a vitamine was bound up in the aetiology of this disease. Our knowledge of vitamines has up to the present depended upon work somewhat limited both in its nature and the number of species of animals investigated, and it was hoped that the use of the dog would not only lead to results more directly applicable to the human being, but also would afford a wider basis for considering the part played by these substances in general nutrition.

This research has centred round calcium metabolism because it is the one object upon which all experimental work on rickets can be focused with the certain knowledge that a solution of the problem will clear up the practical problem of rickets as a disease. A large amount of German research has been carried out with the idea that a deficient calcium intake alone is responsible for rickets. Even now this hypothesis meets with strong support but it will be generally admitted that experimental results on animals and clinical experience are opposed to calcium deficiency as being the main cause of rickets. The problem of deficient intake of calcium has received comparatively little consideration in this research. A sufficiency of calcium has been given in most diets, an amount, that is to say, which will afford the opportunity for the formation of abundant good bone if the other conditions are satisfactory. Whether the body can make full use of the calcium depends on several circumstances and the study of these determining circumstances forms the basis of this investigation.

The material accumulated in this research is great and much of it, especially on the histological side, awaits closer examination. In the course of the work, facts bearing on other diseases have become evident and these, when more completely developed, will be published elsewhere.

II. METHODS OF EXAMINATION

Various methods have been employed for diagnosing and estimating the degree of severity of the rachitic condition, and it is necessary to discuss these methods, more particularly because it is sometimes difficult to correlate the results obtained by the different methods. It is true that as a general rule, a puppy, having the appearance of advanced rickets, will show on radiographic and histological examination typical signs at the ends of the long bones together with the production of osteoid tissue. In addition, also, the calcium content of the bones will be very low. On the other hand, it sometimes happens that the calcium content of the bones may be low when at no stage of the experiment have the external appearance or radiographic results revealed bad rickets. Very occasionally in such cases, there may have been but small external indications of the disease. The chief characteristic of rickets is the defective laying on of calcium salts in the growing bones. This has been emphasized by Pommer (3), Schmorl (4), and other German investigators. This poverty in calcium is revealed by all the methods of examination, but in different ways and at different stages of the

disease, so that each method has its advantages. Absence of or deficiency in calcium salts may be the outcome of many pathological conditions in addition to rickets, such as osteomalacia, pseudorachitic osteoporosis, scurvy, &c., and, although the pathological anatomy of these diseases is still obscure, there is sufficient knowledge accumulated about rickets to place the histological method of examination in a position of supremacy.

The following methods have been used for identifying the rachitic condition :

- (1) External appearance.
- (2) Radiographic examination of the bones during life.
- (3) Histological appearance of the bones.
- (4) Calcium content of the shafts of the bones.

(a) External Appearance.

The appearance of rickets in a dog (Fig. 2) is comparable to that of a rachitic child (Fig. 1). The swollen epiphyseal ends of the bones are unmistakable, more particularly at the growing ends of the radius and ulna. This swelling can be well seen in Fig. 2, which is a photograph of a retriever with rickets. The costochondral junctions also enlarge and, if the dog has a smooth coat, its rickety rosary can often be easily seen. In this work, however, but little reliance has been placed on the swelling of the costochondral junctions, for it has been found to be so variable from animal to animal. A large quickly growing puppy nearly always has more prominent junctions in this position even in a normal state. In other cases, the swelling may be more developed on the inside of the chest, and can only be appreciated after death, although the animal is really rachitic. In very bad cases the costochondral junctions may be drawn in, giving the chest a very deformed appearance. Again, the abdomen is usually prominent in rachitic dogs, and a sulcus corresponding with the well-known Harrison's sulcus of a child is sometimes seen.

As in children, the animal often becomes more lethargic and listless as the rachitic changes develop. The intense interest of the normal healthy puppy in all its surroundings disappears to a large extent, and there is great diminution in its small movements. Often the animal loses its desire to bark, and in this respect resembles a 'good' child with rickets. The muscles are flabby and are unable to contract, to allow the animal to run quickly. Long before the bone changes are sufficiently severe to be an impediment the animal may be incapable of running at any speed. Sometimes the effort to run is lamentable, although the appearance of the puppy would not lead any one to suspect this disability. The power to run has often been used by me to test the relative development of the disease in various members of an experimental series, and, although the method is crude, it is sometimes possible to state with reasonable accuracy how the experiment is proceeding.

The ligaments of rachitic puppies often become slack and this exaggerates greatly the appearance of bending of the legs. Occasionally this happens a week or two after the beginning of the experiment, before any rickets can have developed, and, at that time, such

a change is very misleading. At these early periods I doubt whether it has anything to do with rickets, and it can often be traced to the animal getting wet when very young, and it may appear in animals closely confined. When the ligaments give in this way, often in one leg only, it is impossible to compare the animal affected with the other puppies in the series, as the puppy may be incapable of movement. In the later stage, when the disease is fully developed, the ligaments generally slacken, but then it may be regarded as a more natural rachitic change and is in keeping with the rachitic syndrome. When the disease is fully developed, the bones bend because of their softness due to deficiency in calcium salts. The amount of bending depends not only on the softness of the bones but also on the weight of the animal. The heavier the animal, the more bending will be evident, so that in the case of small light animals there may be little or no bending although rickets is present.

(b) Radiographic Examination of the Bones.

This is an excellent method for following up the development of and recovery from the disease. For a large period of this research it has been customary to radiograph the wrist joint of each puppy at intervals. Changes are seen in the first place at the growing ends of the bones. Often the first indication of rickets is seen in changes in shape of the epiphyseal end of the diaphysis of the ulna. This in normal growth is pointed and ought to fit closely into the corresponding depression of the ulna epiphysis when this is formed. With the development of slight rickets the end becomes more or less rounded, and, when more severe, flat or even concave. These changes can be seen in many of the radiographs which are illustrated in this paper. The flattening of the growing end of bone is the earliest and most sensitive change that can be readily observed. With this flattening the amount of tissue easily pervious to the X-rays and containing deficient calcium between the epiphysis and diaphysis increases. The broader this band of non-calcified tissue the worse is the rickets.

On close examination of the growing edge of cartilage it will often be seen that it has a wavy and indefinite outline in rickets, whereas in the normal animal it is straight and thin. If the disease has started in the puppies at an early age there may be delay in the ossification of the epiphyses. The growing end of a bone in a normal puppy is highly calcified and is more impervious to the rays, so that it generally stands out in sharp contrast to the rest of the bone. In slight cases there may be no other indication of rickets than the absence of this more intense calcification. This point is well seen in Figs. 52 and 54. In Fig. 52 the puppy had had cod-liver oil as its fat, and in Fig. 54 pea-nut oil had been eaten. In neither radiograph would rickets be diagnosed by the ordinary tests of increased zones of uncalcified tissue. It can be easily seen, however, that there is a difference in the intensity of the calcification processes going on in the newly-formed calcified tissue. In Fig. 54 (pea-nut oil) there is no band of increased calcification at the growing end, whereas the cod-liver puppy, Fig. 52, shows this in good contrast. Calcification is more intense in the cod-liver than in the pea-nut oil puppy.

In more advanced cases of rickets, instead of the calcified matrix

appearing in the radiograph as parallel bands at right angles to the growing cartilage, it is irregular and sometimes granular.

Another difference between the normal and rachitic bone is seen in the thickness of the calcified periosteal bone, this difference being more prominent when the experiment has continued for more than three months. It is often very striking and may sometimes appear at an early stage. The calcified periosteal bone in rickets is often thinner, thus giving the appearance of an enlarged medullary cavity. This difference in the amount of compact bone under the periosteum can be seen in Figs. 128 and 129 and in many of the radiographs. If the rachitic diet is only started after the puppy is three or four months old, no epiphyseal changes of note may be evident on radiographic examination. In these cases, however, the difference in the thickness of the periosteal bone between the normal and rachitic animal will stand out prominently.

The question of the size of the medullary cavity is an interesting one. It is certainly often increased because of the thinness of the periosteal bone, but, apart from this, it will usually be found to be abnormally large. The rachitic bone is generally broader, and no doubt some part of this increased diameter of the medullary cavity is due to rickets. On the other hand puppies with the normally broader bones seem to develop rickets more easily—generally, no doubt, because they belong to bigger and more rapidly growing dogs. But it is difficult to say precisely how much of the increased diameter is due to rickets and to what extent the rickets is more intense because of the broader bones. This enlarged medullary cavity and the deficiency in periosteal compact bone may remain throughout the life of the animal even after recovery from the disease, although on recovery the medullary cavity especially at the ends of the bone becomes smaller in diameter (see Exp. 192). In Figs. 91 and 99 can be seen the great contrast in the size of the cavities of bones in a normal (Fig. 91) as compared with the bones of what at one time was a rickety dog, which recovered later on change of diet (Fig. 99). The difference in the amount of compact bone in these cases is also evident. In each case the animal was full grown when the radiographs were taken. It will also be noticed how with recovery the epiphyseal swelling at the growing end diminishes in size and the shaft-ends become thinner (Figs. 97-9).

(c) The Calcium Content of Bones.

It has been usual to estimate the calcium in the shafts of the femurs of each experimental animal, as long a piece of the shaft being taken as possible in each case. The method used for estimating the calcium was that described by Cahen and Hurlley (7). The bone was weighed immediately on being cut out, the muscle, &c., attached to it having been dissected off. This weight is that of the 'fresh bone'. It was then heated at 105° to 110° C. for about eight hours until the water was driven off and weighed again. The second weight is that of the 'dry bone'. Many of the results are given as percentage CaO in the fresh and dry bones. It will be seen on examining the figures that sometimes there is a discrepancy between the results, the calcium percentage in the dry bone appearing high when

the corresponding percentage in the fresh bone is low. This discrepancy can often be explained. In some cases the animals have gone off their food and lost weight continuously for a time before being killed. When this happens, the marrow fat diminishes and often disappears entirely. When the bone is weighed in the fresh condition, the medullary cavity, instead of containing fat as it usually does, is full of blood or other tissue fluids. When dried, this fluid evaporates, but drying does not get rid of the fat, so that, whereas the former bone loses a lot of weight by drying, the latter loses much less. Consequently, when there is no marrow fat, the percentage CaO in the fresh bone may be very low, but when reckoned in terms of the dry bone it will be much higher. It is clear that the percentage CaO content of the fresh bone is the more reliable figure for comparative purposes.

The calcium content of the bones is a useful indication of the intensity of rickets when controlled by histological and radiographic methods, but it is necessary to remember that the figures cannot be used indiscriminately and several points of importance must be considered.

(1) The percentage amount of calcium in the shaft of a bone depends, even in normal dogs, on the age and breed of the animal. A young animal of, say, four months old may only have about 11 per cent. CaO in the fresh bone, whereas at six months the percentage may be 14 to 16. The percentage CaO in the fresh bone of an adult dog is 24 or more. In comparing figures, therefore, the animals ought to be of similar age. It is obvious that the longer the experiment continues, the greater will be the difference in calcium content between normal and rachitic dogs.

(2) The wider the medullary cavity and the greater the cross section of the bone, the lower will be the calcium percentage content. It has been pointed out above that rickets is more frequently associated with dogs having bones with wide medullary cavities, but it cannot be accepted that all this widening is due to rickets, because the disease appears to attack more readily those puppies, even in the same family, with the broader bones. The broader the bone the greater is the amount of calcification necessary to keep the growth normal. In using the calcium results, therefore, as a measure of rickets, this difference in the cross section of bones must remain a difficulty, and it can only be surmounted by having the dogs as similar as possible in comparative experiments and, even then, by not arguing too precisely from small differences in results. As a general rule, the differences in calcium content of normal and rachitic dogs is so great, more especially if the experiment has continued over a period of four months or more, that the question of size of bone is of small importance, but, in the more refined experiments where small differences are being examined, it is a point worth due consideration.

(d) Histological Examination of Bones.

Schmorl (4) states that only those experimental results on rickets in animals are worth consideration when the chemical examination of the bones is controlled by a knowledge of their pathological anatomy. With this I agree, although there appears to be no

definite views as to what is the true histological appearance of rachitic bones in dogs. That there must be some difference between human and canine rickets from the point of view of minute anatomy is certain, even if only because of the difference in the time relations of the disease in the two types of animals. According to my experimental results, to produce typical rickets in hitherto normal puppies the treatment ought to start in the first three months of life. In children typical rickets does not often develop after the age of three or four years, and is generally found under the age of two.

Many of the changes observed at the epiphyseal ends of bones in rickets can be produced experimentally in puppies by other conditions than those conducive to rickets. The hypertrophy of the proliferating cartilage, the invasion of the cartilage by marrow vessels, the absence of calcification at the cartilage-bone zone can also be produced by deprivation of calcium salts and by feeding with phosphate-poor food (Heubner (8)). The other histological appearances are not, however, the same as in rickets, so it is evident that no reliability can be placed on the histological appearance of endochondral ossification as the final test of rickets, although as in the case of children, these changes at the epiphyseal ends are a useful guide. According to Schmorl (4), endochondral changes similar to those seen in rickets can also be produced in man by syphilis, Barlow's disease, and traumatic separation of the epiphysis. Sinceradiographic examination reveals for the most part changes of calcification at the epiphyses, the above facts make its limitations obvious. As most of the figures given in this publication are radiographs, it is necessary to add that the experiments have been controlled histologically and chemically.

The presence of osteoid tissue is the crucial test of rickets in children as first pointed out by Pommer (3). When, therefore, excess of osteoid tissue is found in puppies' bones, until the subject of rickets in dogs has been more completely worked out, it must be accepted that a rachitic condition is present, even when there are other complicating bone changes. When Dibbelt (9) produced an excess of osteoid tissue in the bones of puppies fed on horse flesh and carbohydrate, Schmorl describes it as 'etwas Akzidentelles' because there were also present osteoporosis and abundant osteoblasts lying on the osteoid layers. In addition also there was evidence of abnormally great bone absorption in the excessive number of osteoclasts. There would appear to be no great difficulty in imagining a combination of rickets and osteoporosis as the diagnosis of the condition produced by Dibbelt.

Many histological methods have been used in this research for demonstrating the presence of osteoid tissue.

These include

1. Fixing and partially decalcifying in Müller's fluid and subsequently staining
 - (a) in ammonia carmine (Pommer). Figs. 123, 124.
 - (b) in silver nitrate and eosin. Figs. 125, 126, 127.
 - (c) in methylene blue and eosin.
2. Schmorl's thionin method. Fig. 122.

3. Weil's method, which consists in fixing the tissue in mercuric chloride, staining with borax carmine, impregnating in balsam, and grinding down the section. The bone is not decalcified by this method. Figs. 118, 119.
4. Cutting small pieces of bone—not previously decalcified—and staining by various methods. The silver nitrate-eosin staining method gives good results.

Microphotographs of bones stained by these various methods are shown in Figs. 114-27.

When there is osteoid tissue present, it will usually be found that the calcified bone of the trabeculae is also imperfectly formed. The cells of this imperfect bone are swollen, irregularly placed, and sometimes scarce, and are in striking contrast to the cells of true bone which have a squeezed appearance and are more regularly arranged. These differences are evident whatever method of preparation and staining is adopted. (Compare microphotographs, Figs. 120 and 121.) The fibrillar appearance of osteoid tissue can also be seen (Fig. 124).

Macroscopically also osteoid tissue can often be easily recognized especially in advanced rickets. It will be found in such cases that the compact bone forming the lamellae of the skull bones is thick and soft and cuts easily with a scalpel. Sometimes the bones are so soft that they can be bent with the fingers.

As regards the possibility that the pathological condition produced in the puppies is pseudo-rachitic osteoporosis or that this condition is a complicating factor, it may be stated that osteoporosis is not often a feature of the bones. In one or two experiments where the effect of acidic caseinogen was tested, there was some osteoporosis in addition to rickets. These were exceptional cases. Osteoporosis would not be expected as the puppies received a good supply of calcium salts in their diet.

The number of osteoblasts and osteoclasts indicating the extent of laying down and absorption of bone varied greatly in different experiments, but in general there was a great diminution of these cells in the bones of rachitic animals. On the other hand, even without any advanced rachitic development, cessation of growth may be accompanied by a striking diminution of these cells (Exp. 300). In another member of the same group (Exp. 299) where the growth was vigorous but rickets not pronounced, the osteoblastic and osteoclastic activity was very great.

The pathological anatomy of rickets as it appears in the puppies of this experimental work may be said to be strictly comparable to that met with in human rickets in so far as the histological appearance of the endochondral ossification is concerned. There is also abundant osteoid tissue present while osteoporosis, except in certain cases, is absent. Again, in most of the experiments the incidence of rickets is associated with diminished osteoblastic and osteoclastic activity.

Although I have no doubt that the condition developed in the puppies is comparable to rickets in children, I agree with Schmorl's plea that it is essential that the pathological anatomy of the bones of animals should be closely reviewed, because there are so many different factors capable of influencing the formation of bone. To

me the subject is one of extraordinary difficulty, and I should like to acknowledge the assistance I have received from Professor S. G. Shattock in considering this side of the work.

(e) **General Consideration of Methods.**

I have stated above that, although on the whole there is general agreement between the results obtained by the various methods employed in this research, yet there are occasions when this is not so. For instance, in a few cases the calcium content of the bones would point to very pronounced rickets and yet the radiographic and histological results indicate that at no period of the experiment has the disease been advanced. Some part of this disagreement can be explained. In puppies, the rachitic symptoms of enlarged epiphyses and the changes associated with this enlargement which can be detected by radiographic and histological methods, develop only in young animals under the conditions tested in this research. If the experiments begin when the puppies are at least 3-4 months old no obvious rickets may develop. If, however, the dietetic conditions conducive to rickets be maintained in these older animals, although the usual outward signs of rickets are absent, it will be found that calcification of the shafts of the bones is defective, a fact which can be detected by the radiograph and better still by determining the calcium content of the bone shafts. In any particular animal therefore, if the diet has only slight rickets-producing effect, the animal may tide over the earlier part of the experimental rickets and escape enlarged epiphyses and other gross changes of endochondral ossification. That, however, the growth changes in the bones are not normal can be seen when the calcium in the bone shaft is estimated and found to be much below the control. Several instances in the course of this work have been met with where the rachitic symptoms have appeared small and at the same time the calcification processes have been defective. In Figs. 128 and 129 is seen the relative thickness of the periosteal bones when cod-liver oil and rape-seed oil were eaten. Neither puppy had rickets at death according to the radiographs but there is a great difference in the thickness of the bone and in the histological picture.

On the whole the radiographic method is valuable for detecting rickets, especially in the earlier stages, but there are occasions when it fails, together with the deductions made from external appearance. In all these cases, the calcium content of the bone shaft in addition to the minute anatomy give useful information as to the failure or success of the experimental methods employed in producing abnormal or normal bone. I wish to emphasize that the swollen epiphyses and the apparent excessive production of tissue at the growing ends is a process which takes place in the younger puppies, whereas a deficiency in calcification is a process which may continue over a long time, probably in fact for the whole period of bone growth.

The importance of these facts can be readily seen as they apply to man. Babies under two are specially liable to rickets and after this age the disease in an active form is much rarer. This does not

mean that calcification processes in bones and teeth in later years are necessarily normal.¹ The comparative rareness of the classical signs of rickets in children over two years of age is very misleading and it cannot be too strongly emphasized that in spite of their normal appearance, the formation of the bony tissues may be extraordinarily defective so long as growth continues.

This point has a still greater significance when it is remembered that the calcification of the teeth is wrapped up with the question of rickets and that the complete elimination of the latter would go a long way to settling the problem of defective teeth in civilized man. This investigation on the teeth is being worked out by May Mellanby (10), who has already shown that perfect and imperfect calcification of the teeth is a nearly related problem to similar changes in the bones.

While, therefore, I would be the first to admit that rickets is not alone a deranged calcification of bones, as an objective method of study the chemical method presents the advantages of quantitative measure lacking in the histological and radiographic methods.

It is evident that the calcification processes of bones can be deranged by many other causes than those responsible for rickets, and that to place too much weight on the calcium content may lead away from the particular disease and involve the investigator in a worse morass than rickets itself. This can only be met by constant control of the experimental conditions and the use of histological methods. It is true that, in some of the earliest experiments, the rachitic changes were possibly associated with scurvy but, in all the later experiments upon which I place most reliance, orange juice has formed an element of the diet. The fact that I hardly ever see any indication of pain in the most rickety puppies is evidence that scurvy has been eliminated.

The further danger of mixing up rickets and pseudo-rachitic osteoporosis has already been dealt with.

III. EARLIER EXPERIMENTS

Anybody familiar with the large number of hypotheses that have been advanced to explain the aetiology of rickets, must be aware of the difficulties under which the beginning of a new investigation of this disease is made.

The only outstanding and definite idea at the commencement was the necessity of carrying on the experiments with animals capable of developing the disease. For it is a commonplace that this experimental method offers the best means for making definite advance. We have seen for instance that Eijkmann's (11) discovery of 'poly-neuritis gallinarum' was the beginning of the investigations which ultimately led to the solution of the 'beri-beri' problem. Holst and Fröhlich's (12) work on scurvy in guinea-pigs quickly

¹ In this connexion it is well to remember that of 99 children who died between the ages of 2 and 4 years, and whose bones were examined histologically by Schmorl (4), 81 showed signs of rickets. In 15 of these cases the disease was active, in 17 healing, and in 49 healed.

brought to light the aetiology of scurvy in man and placed that disease on a definite scientific basis.

There was, therefore, reasonable certainty that, if the conditions under which an experimental animal develops rickets could be found, the problem would then be fairly accurately defined and made easier. Fortunately the experimental animal was ready to hand, for it has long been recognized that puppies develop rickets. What to do with the puppies in order to produce the disease was unknown to me, because all the hypotheses that have been advanced to explain rickets in children could likewise be offered in explanation of a similar disease in puppies.

At the beginning of this investigation a hypothesis discussed by clinicians was that depending upon the work of Findlay (13) in which he showed that confined puppies developed rickets while other puppies living on the same diet, when allowed exercise, remained normal. According to Findlay the disease of rickets in children could be explained by deficiency of exercise. Experiments, which will be described later in the paper, were prepared in order to test the exercise hypothesis. It was soon evident that, although exercise might be a factor of importance, yet it could not be the primary factor responsible for the disease. It was shown, for instance, that puppies could be confined under very close conditions and yet, if well fed during this period, the disease did not develop. Exp. 198, Figs. 89 and 90; Exp. 350, Fig. 113.

In order to get a line for research a large number of preliminary experiments were carried out with the object of testing various hypotheses, experiments many of which gave no result worth following up. It seemed for instance that reduced oxidation changes in the animal's body might play an important part in the development of the disease. On this basis attempts were made to produce local changes in bones as seen in rickets by, for instance, removing or tying the lymphatic vessels of one limb. It was thought that a local operation of this sort which would interfere with the removal of waste products from the limb, might at the same time reduce the oxidation changes in this way. All the experiments performed on these lines were negative in their results.

In other experiments performed with the same object the attempt was made to produce lymphatic stasis more or less complete throughout the body by tying the thoracic duct just as it enters the venous circulation. In these experiments no rachitic changes resulted that could definitely be ascribed to the cessation of lymph flow.

Again, in order to reduce processes of oxidation, quinine was given to other puppies. These experiments also led to no positive results.

During the course of the above-mentioned work, experiments of a tentative nature were being carried out to see how the disease could be effected by diet. In the first experiment made, a puppy was fed on boiled horse flesh without fat. After about ten weeks of this diet the animal developed a rickety appearance. It seemed certain, however, from the general condition of the animal that scurvy was associated with rickets. There was, for instance, obvious pain in walking. After being anaesthetized, the diagnosis of scurvy

was made certain by the haemorrhages present. It was found at this time that if puppies, in addition to the meat, received a substantial quantity of milk each day, they remained in good health.

While testing the effect of varying salts in the diet, a mixture consisting of milk and equal parts of oatmeal and rice was used as the basal diet because of its low sodium content. It was found that on a diet of oatmeal, rice, and milk puppies ultimately developed rickets when the milk was limited to about 200 c.c. per diem. The addition of sodium chloride did not influence the development of the condition. The addition, on the other hand, of a small quantity of potassium chloride resulted in severe malnutrition and death.

A large number of experiments were made in which this diet of milk, oatmeal, and rice was used as the standard and, in all cases under laboratory conditions, the animals developed rickets. The treatment which prevented the disease was an increase in milk consumption to, say, $\frac{1}{2}$ litre a day. When the oatmeal and rice were replaced by ordinary white bread, other conditions being the same, rickets also developed.

The drawback to the use of this diet was that the period necessary for the production of the disease was long, often 4-6 months, and, except in puppies of a large rapidly-growing type, the bony changes and other symptoms were not as strongly developed as was desirable from an experimental point of view.

The natural question that arose at this stage was, what was the factor associated with the large milk intake that prevented the development of rickets? The answer that suggested itself, as may well be imagined in consequence of the results obtained by Bland Sutton on the effect of cod-liver oil when given to young lions in the Zoo, was that the fat of milk contained some inhibitory factor. It appeared in other words as if the explanation of rickets was to be found in the theory that a deficient fat intake was the main responsible factor.

In order to test this point the fat of the milk was removed by skimming, and it was noticed that the animals developed rickets more rapidly. At this stage of the work a concoction called 'Marylebone cream' was being distributed in the Infant Welfare Centres in London as a curative agent for rickets. 'Marylebone cream' was then an emulsion of linseed oil, and as a matter of interest the effect of linseed oil on the development of the disease was tested. The cream was removed from the milk by skimming and replaced by an equivalent quantity of linseed oil, and it was soon discovered that linseed oil did not possess the anti-rachitic effect as did the milk fat.

Before this period McCollum's (14) work on the distribution of Fat-soluble A vitamine was published. The results of experiments carried out with linseed oil as opposed to butter naturally led to the suggestion that the Fat-soluble A vitamine played some part in the aetiology of the disease.

It seemed advisable to investigate the action of the other two vitamins, viz. the water-soluble and the anti-scorbutic, on rickets. In order to test the effect of the water-soluble vitamine, yeast was added to a diet of milk, bread, and linseed oil (in these early experiments

the milk was only skimmed and not separated). The effect of adding the yeast was that the animals appeared to thrive better, but there was quite obviously no anti-rachitic effect, that is to say, the water-soluble factor had no influence in preventing rickets. In other experiments the effect of the anti-scorbutic vitamine was tested. To a diet of milk, bread, yeast, and linseed oil, the juice of a quarter of an orange daily was added. The addition of the orange juice again seemed beneficial for the general health of the animals but once more did not prevent the development of the disease. It was in fact evident that the deficiency of anti-scorbutic and anti-beri-beri factors did not explain the aetiology of rickets.

Large numbers of experiments were carried out in the endeavour to determine the part played by other dietetic factors. For instance, to a diet of bread and limited milk, substances like malt extract, cod-liver oil, and other forms of fats such as suet, pea-nut, coconut oil, and cotton-seed oils, also meat protein, meat extract, casein, were added and their effect on the development of rickets tested.

These early experiments were crude, but the results were more or less in keeping with those obtained by the later more refined methods. For instance, cod-liver oil and suet had pronounced anti-rachitic action, meat also had a preventive effect to some extent, and malt extract had an anti-rachitic action when eaten in large quantities. Quantities less than 20 c.c. a day appeared only to have slight inhibitory effect. Some of these earlier results have been published (1).

It may be of interest to add that the experimental work had continued for about two years before good evidence of differences in the anti-rachitic action of fats was obtained.

IV. THE EFFECT OF DIET ON THE PRODUCTION OF RICKETS

(a) The Effect of Various Oils and Fats on the Development of Rickets.

We have seen in the foregoing section how the earlier work led step by step to the evolution of a diet which produced rapid and marked rickets in well growing puppies. This diet consisted of :

- Bread *ad lib*.
- Separated milk 175 to 250 c.c.
- Yeast 5 to 10 gm.
- Orange juice 5 c.c.
- Sodium chloride 1 to 2 gm.
- Linseed oil 10 c.c.

I now propose to deal with the effects of other oils, and to describe the experimental work, upon which is based my previously published deduction, that there is great difference in the anti-rachitic effect of the different oils tested. When alluding to this part of the work in previous publications, I have insisted that the classification of fats into a definite order as to the amount of anti-rachitic factor present in each, must be of a tentative nature. This is not because

of the lack of work on the point, but because of the great difficulty of carrying out quantitative experiments so as to give definite and precise information. Facts of this nature are only brought to light by working on animals of definite age, size, rate of growth and breed, keeping all factors in the diet except the fat constant.

In the earlier experiments on the action of fats in rickets, I had not recognized that the amount of bread eaten by the puppies was a crucial point. Up to this time all factors in the diet were controlled except the bread, which was varied according to the appetite of the animal. This fact deprives the earlier work of some of its quantitative accuracy. Nevertheless, definite results were obtained which agreed, in the main, with the experimental results obtained later when all the elements of the diet were eaten quantitatively.

Even with full knowledge as to the conditions which must regulate these experiments, it is very difficult to carry out perfectly a comparable series. The puppies will probably eat their food for ten weeks or so. Then the defective diets begin to have a real influence on the health of the animals, and one by one they leave some food uneaten. If the experimenter decides to get a result at this stage by radiograph, then the animals will have to be anaesthetized, and this procedure will increase the number of puppies not completely finishing their diets. I shall discuss elsewhere this difficulty of recovery from anaesthesia in the case of dogs on deficient diets, as it is apparently a point of practical importance (p. 72).

Up to the present, when dealing with different fats I have not succeeded in getting a completely satisfactory quantitative experimental series beyond about the eleventh week of dieting. At the end of this period, radiographic and histological differences are well brought out, but in order to obtain good differences in the calcium content of the shafts of the bones, the experiments ought to continue for fourteen weeks or longer. In spite of these difficulties, a fair insight has been obtained as to the relative anti-rachitic power of the various fats, but there is still need for much work on the subject. There is great variation in method of manufacture of oils and fats, even from the same type of seed, and there remains the problem of ascertaining the effect of the manufacturing processes on the anti-rachitic vitamine. Speaking generally, however, this difficulty has not led to the great variation in results that might have been expected, possibly because the types of oil examined have been of the same standard. In the first experiments olive oil appeared to hold a higher position in content of anti-rachitic vitamine than was obvious in later experiments, but this difference must, I think, have been due to the smaller amount of bread eaten.

In most of the later series of experiments, meat was put into the diet in order to induce the puppies to eat up completely each day's ration. This plan ensures more uniform results in comparable experiments, but has the drawback of reducing the rachitic changes. It is a question of choice between producing very bad rickets but less uniformity for comparison and more uniformity and less rickets.

I shall now proceed to give an account of some of the numerous

experiments on fats that have been carried out. Unless otherwise stated, the animals in each series belonged to the same family.

In referring to the histological condition of the bones 'slight rickets' means less abnormality than 'some rickets', and both of these less than 'rickets'.

COMPARISON BETWEEN LINSEED, BUTTER, OLIVE, AND COD-LIVER OILS.

General Diet :

Bread *ad lib.*
Yeast 10 grm.
Separated milk 175 c.c.

Age at commencement, 8 weeks.

<i>No. of experi- ment.</i>	<i>General Diet.</i>	<i>Duration.</i> weeks.	<i>Initial</i>	<i>Final</i>	<i>Gain.</i> gm.	<i>CaO in femur shaft.</i>		<i>Histology results.</i>
			<i>weight.</i> gm.	<i>weight.</i> gm.		<i>Dry.</i> %	<i>Fresh.</i> %	
145	+ linseed oil, 10 c.c.	17	1,830	3,605	1,775	21.60	12.35	Rickets
146	+ butter, 10 grm.	17	1,920	3,765	1,845	26.95	15.89	Normal
147	+ olive oil, 10 c.c.	17	1,445	2,645	1,180	23.79	13.22	Some rickets.
148	+ cod-liver oil, 10 c.c.	17	1,735	3,890	2,155	27.78	16.51	Normal.

These puppies were wire-haired terriers of a slowly growing type. The histological examination showed that only one, viz. 145, the linseed oil puppy, had well-developed rickets; 147, olive oil had some rickets, whereas 148, cod liver oil, and 146, butter, were normal. It is interesting to note, what has been corroborated throughout, that cod-liver oil is better than butter. In these experiments the cod-liver oil dog has a higher percentage of calcium in the shaft of the femur than the dog which had butter. Another point to note about this particular experiment is that the cod-liver oil dog grew most rapidly and the olive oil dog the least rapidly. This difference was largely due to the better appetite of cod-liver oil dog, so that it ate more bread. The olive oil puppy ate least bread and this is probably the reason why it only developed slight rickets (see p. 42). The cod-liver oil puppy, on the other hand, remained normal in spite of eating most bread. Note also the comparatively good growth of 145 on the linseed oil. According to accepted teachings the diet eaten must have contained a minimum of Fat-soluble A, and in spite of this deficiency, good growth resulted.

COMPARISON BETWEEN LINSEED AND COD-LIVER OILS.

General Diet :

Bread <i>ad lib.</i>	Salt 1 grm.
Yeast 5 grm.	Separated milk 175 to 250 c.c.
Orange juice 3 c.c.	Meat 10 grm.

Puppies—cross between collie and terrier. 9 weeks old at commencement of experiment.

General Diet with Various Oils.

No of experi- ment.	General Diet.	Duration. weeks.	Initial	Final	Weight	CaO in		Histology results.
			weight. gram.	weight. gram.	after 3 mths. of diet. gram.	femur shaft. Dry.	Fresh.	
176	+ linseed oil, 10 c.c. . + 175 c.c. sep. milk .	18	1,705	2,805	5,035	—	7.68	Bad rickets.
177	+ 10 c.c. linseed oil .	18	1,360	2,590	4,155	—	9.56	Bad rickets.
178	+ cod-liver oil, 5- 7.5 c.c.	18	1,750	5,525	4,250	—	12.5	Practically normal.
179	+ cod-liver oil, 10- 15 c.c.	23	1,910	6,140	4,325	—	13	Normal.

The difference in the calcium content of bones in these dogs is striking, and is in conformity with the rickety appearance of 176 and 177 and the normal appearance of 178 and 179. That is to say the linseed oil puppies developed bad rickets, whereas the cod-liver oil animals remained normal, even in the case of 178, where the oil eaten was only 5 to 7.5 c.c. per diem. Notice also that 176, which received as much as 350 c.c. of separated milk each day, developed bad rickets. Both the rachitic animals 176 and 177 became ill and lost a great deal of weight during the last three weeks of the experiment. The radiographic appearance of the wrist-joint of these puppies are shown in Figs. 3, 4, 5, 6. Needless to say, all conditions other than those described were constant, and it is difficult to believe that any factor other than differences in the oil eaten could have been responsible for the great difference in the results obtained.

COMPARISON BETWEEN OLIVE, LINSEED, COTTON-SEED, BABASSU, PEA-NUT, AND COD-LIVER OILS.

General Diet :

Bread *ad lib.*

Yeast 5 gram.

Salt 1 gram.

Orange juice 3 c.c.

Separated milk 175 to 250 c.c.

No meat in diet.

Animals 6 weeks old at the start of the experiment.

General Diet with Various Oils.

No. of experi- ment.	General Diet.	Duration. weeks.	Initial	Final	Gain. gram.	CaO in		Histology results.
			weight. gram.	weight. gram.		femur shaft. Dry.	Fresh.	
185	+ olive oil, 10 c.c. .	7	1,495	2,500	1,005	—	7.3	Slight rickets (7 wks.).
186	+ linseed oil, 10 c.c. .	7	1,325	2,470	1,145	—	6	Rickets (7 ,,).
187	+ cotton-seed oil, 10 c.c.	16	1,335 Max. after 9 weeks,	1,900 2,750	?	—	7.6	Rickets (16 ,,).
188	+ babassu oil, 10 c.c.	7	1,240	1,725	485	—	6.55	Bad rickets (7 ,,).
189	+ pea-nut oil, 10 c.c.	29	1,350 Max. after 22 weeks,	4,000 5,095	—	—	9.77	Some rickets (29 ,,).
190	+ cod-liver oil, 10 c.c.	24	1,320	6,550	5,230	—	14.4	Practically normal (24 ,,).

Of these puppies 185 and 186 were killed by the anaesthetic when the X-ray photographs were taken, and 188 died of intestinal obstruction. 185, 186, 187, and 188 developed rickets at an early age. Radiographs of 186 and 187 were taken seven weeks, 189 and 190 ten weeks after the beginning of the experiment (Figs. 7, 8, 9, and 10). It will be seen that 190 is normal and that 186 and 187 are distinctly rachitic, 186 (linseed) being worse than 187 (cotton-seed), and 187 (cotton-seed) worse than 189 (pea-nut). 188, the babassu oil dog, also developed severe rickets, but the radiograph of this puppy has been mislaid. The only puppies that lived any length of time are 187 (cotton-seed), 189 (pea-nut) and 190 (cod-liver oil). As regards the anti-rachitic action of the fats, the radiographs indicate the following order :

1. Cod-liver oil.
2. Pea-nut oil.
3. Cotton-seed oil.
4. Linseed oil.
5. Babassu oil.

Weight (after 7 weeks of diet).

185	2,500 grm.	Olive oil.
186	2,470 "	Linseed oil.
187	2,290 "	Cotton-seed oil.
188	1,725 "	Babassu oil.
189	2,190 "	Pea-nut oil.
190	3,150 "	Cod-liver oil.

Here again the cod-liver oil puppy (190) has grown most rapidly, but even in the case of the vegetable oils, which are supposed to contain no Fat-soluble A, good growth is evident. As regards those puppies in this series whose life extended over a larger time, viz. 187, 189, and 190, it may be said that the cod-liver oil (190) remained normal throughout the experimental period, cotton seed (187) died at the end of 17 weeks having definite rickets, pea-nut oil (189) also showed slight signs of rickets in the subsequent radiographs that were taken, but these disappeared with the development of curative changes, and the dog remained in external appearance almost normal throughout.

The deductions to be made from this series of experiments, which it will be observed has been divided up into two portions, are that cod-liver oil stands by itself in its anti-rachitic action, and that of the vegetable oils, pea-nut oil is the best, cotton-seed oil next, and olive oil, babassu, and linseed oil are the worst. The results are in agreement throughout whether radiographs, histological appearances, or calcium content of the bones are taken as the measure of the development of the disease. The superiority of cod-liver oil over all other fats tried in promoting calcification of bone is in harmony with the earlier observed fact that it holds a pre-eminent position in its power to convert a metabolic negative calcium balance into a positive one. (Orgler (36), Schabad (37).)

COMPARISON BETWEEN SUET, LARD, BUTTER, AND BABASSU OIL.

*General Diet:*Bread *ad lib.*

Orange juice 3 c.c.

Yeast 5 grm.

Separated milk 200 to 250 c.c.

Salt 1 grm.

Meat 10 grm.

Terrier puppies of same family 6 weeks old at the start of experiment.

General Diet with Various Fats.

No. of experi- ment.	General Diet.	Duration. weeks.	Initial weight. gram.	Final weight. gram.	Gain. gram.	CaO in femur shaft.		Histology results.
						Dry.	Fresh.	
						%	%	
180	+ suet, 10 grm. . .	20	840	4,285	3,445	—	14.4	Normal.
181	+ lard, 10 grm. . .	20	1,075	3,965	2,890	—	9.6	Slight rickets. Soft bones
182	+ butter, 10 grm. . .	19	1,140	5,800	4,660	—	12.5	Some rickets.
183	+ babassu oil, 10 grm.	19	1,460	2,920	—	—	6.6	Bad rickets.
			Max. after 10 weeks, 3,920					

Of this series of puppies the only one that developed obvious rickets, as far as external appearance was concerned, was 183, that is the babassu oil dog. The bones of this animal contained the least calcium and were very soft.

When the radiographs are examined some unexpected results are to be observed. It will be noticed, for instance, as regards the calcium content of the bones, that in the case of the butter dog the bones were much harder than in the case of 181, i.e. the lard puppy. Yet the radiographs taken three months after the beginning of the experiment show that the butter dog had developed obvious rickets, whereas the lard puppy showed no signs of the disease. Radiographs of 180, 181, 182, and 183, taken 14 weeks (suet 18 weeks) after the beginning of the diet, are shown, and it will be seen that 180 (suet) and 181 (lard) are normal, 182 (butter) has some rickets, and 183 (babassu oil) has very bad rickets (Figs. 11, 12, 13, and 14).

These results are of importance because of their inharmonious nature, the butter dog having hard bones and defective endochondral ossification, while the lard dog has soft bones and almost normal epiphyseal growth.

Thus it is apparently possible to get these two factors divorced from each other, although as a general rule they run side by side. The question will be further considered later, but, in the meantime, the difference in the rate of growth between the two puppies under discussion (181 and 182) may be pointed out. 181 (lard) only gained 2,890 grm. during the period in which 182 (butter) gained 4,660. The butter dog laid on a great deal of fat during the experimental period.

PALM-KERNEL OIL (CRUSHED AND EXTRACTED).

*General Diet:*Bread *ad lib.*

Orange juice 5 c.c.

Separated milk 250 c.c.

Salt 1 grm.

Yeast 5 grm.

Meat 5 to 10 grm.

Puppies 7-8 weeks old at beginning of experiment.

No. of experiment.	General Diet	Duration. weeks.	Initial weight. gm.	Final weight. gm.	Gain. gm.	CaO in femur shaft.		Radiographic results after 9 weeks.
						Fresh. %	Dry. %	
220	+ 10 grm. crushed palm-kernel oil	35	1,585	9,900	8,315	14.7	19.3	Very bad rickets.
221	+ 10 grm. crushed palm-kernel oil	35	1,100	4,760	3,660	15.6	20.0	Slight rickets.
223	+ 10 grm. extracted palm-kernel oil	35	1,050	5,000	3,950	14.6	18.0	Bad rickets

For the last six months of experiment 220 received 500 c.c. whole milk, 221 and 223 remaining on the original diet throughout. All showed curative changes.

In nine weeks 220 had developed most severe rickets (the worst form of rickets that had been met with at this stage of the work), 221 in the same period showed some slight indications of rickets, whereas 223 was fairly bad, intermediate between 220 and 221. It would appear that the differences in the condition cannot be accounted for by any difference there may be between the crushed and the extracted palm-kernel oil, because 220 on the crushed palm-kernel oil is the worst, whereas 221, eating the same oil, has the least rickets, 223 on extracted palm-kernel being intermediate between the two. The only other variable in these experiments was the amount of bread eaten. 220 was a heavier dog to begin with, ate more bread, and grew much more rapidly than either of the other two puppies. The differences in the rate of growth can be seen in Fig. 23. It is probable that the difference in initial size and the varying rate of growth explain the different degrees of severity of the disease in these three cases. This fact will be referred to elsewhere in the paper when the effect of varying the amount of bread in the diet is dealt with (p. 42).

Another point of interest concerning this particular family is the self-cure which took place in both 221 (Figs. 21, 22, 25) and 223 (Figs. 18, 19, 26), although all conditions as regards diet and environment remained constant.

220, which had the very pronounced rickets, was put on to a whole milk diet and subsequently cured, although its deformities were so bad that these were only partly improved (Figs. 15, 16, 17, and 24). It would appear from this series of experiments that palm-kernel oil is deficient in the anti-rachitic factor.

Radiographs taken after nine and thirteen weeks of the experimental diets are shown (Figs. 15, 16, 18, 19, 21, and 22).

THE EFFECT OF HEAT ON COD-LIVER OIL.

Exp. 229, 231, 232. This experiment was made in order to test the effect of heat on the anti-rachitic action of cod-liver oil.

General Diet :

Bread <i>ad lib.</i>	Orange juice 5 c.c.
Yeast 5 grm.	Salt 1 grm.
Separated milk 250 c.c.	Meat 10 grm.

Puppies were a cross between half-bred spaniel and Airedale and were seven weeks old at the start of the experiment.

Confined to kennels (indoors) throughout greater part of experiment.

General Diet with Cod-liver Oil heated and unheated.

No. of experi- ment.	General Diet.	Duration. weeks.	Initial weight. gram.	Final weight. gram.	Gain. gram.	CaO in femur shaft.		Histology results.
						Dry. %	Fresh. %	
229	+ cod-liver oil unheated, 10 c.c.	14	2,010	3,590	1,580	24.3	12.3	Normal
231	+ heated 4 hours at 120° C., 10 c.c.	16	2,710	5,380	2,670	21.4	10.4	„
232	+ heated 2 hours at 120° C., 10 c.c.	16	1,675	5,000	3,325	24	12.6	„

The radiographs taken at intervals show 229 (Fig. 27) to be normal. In 231 (Fig. 28) there is very slight flattening of the diaphyseal bone at the epiphysis after two months of the diet, whereas 232 (Fig. 29) is practically normal. It seems in fact that the cod-liver oil deteriorated to a very slight extent in so far as its anti-rachitic properties are concerned, by the prolonged heating at 120° C. for four hours, but that heating only for two hours seemed to have no effect in destroying this factor. The heating of the oil took place in a closed flask, plugged with cotton wool.

The calcium content of the bones corroborates the radiographic results, for it will be seen that in 231, where the heating went on for four hours, the calcium oxide in the fresh bone was reduced to 10.4 per cent., whereas in the other two puppies it was 12.3 per cent. and 12.6 per cent. respectively. The minute structure was normal in all cases.

The results obtained in this series are not striking, and it would be necessary to repeat the experiments under better conditions before more accurate deductions can be made. The fact that even 231 shows but slight rachitic changes makes it certain that this factor is very resistant to direct heat. Other results will be recorded later which show that in the case of butter, if oxidative changes are allowed to go on while heat is being applied, then the destruction of the factor is more rapid. 231 grew most rapidly during the earlier period, so that it is just possible that the very slight rickets that can be observed in this dog may be due not so much to the destructive action of the heat as to the more rapid growth. Radiographs of 229, 231, 232, are shown in Figs. 27, 28, and 29.

In first ten weeks of experiment :

229	increased from 2,000 to 3,600—gain 1,600 gram.
231	„ 2,700 to 5,800— „ 3,090 „
232	„ 1,700 to 4,120— „ 2,420 „

COMPARISON BETWEEN COCO-NUT OIL AND HYDROGENATED FAT.

Exp. 264, 265, 266, 267. This experiment was started when the puppies were older, i.e. when they were twelve to sixteen weeks old. They were not of the same litter.

General Diet :

Bread <i>ad lib.</i>	Orange juice 5 c.c.
Separated milk 200 c.c.	Salt 1 gram.
Meat 10 gram.	

General Diet with Different Fats.

No. of experiment.	General Diet.	Duration. weeks.	Initial weight. gram.	Final weight. gram.	Gain. gram.	CaO in femur shaft.		Histology results.
						Dry. %	Fresh. %	
264	+ coco-nut oil, 10' gram.	21	1,240	3,080	1,840	27.4	20	No rickets, hard bones.
267	+ coco-nut oil, 10 gram.	21	4,240	5,550 Max. after 10 weeks, 6,700	1,310	26	19	No rickets, hard bones.
265	+ hydrogenated fat, 10 gram.	21	2,605	5,170	2,575	19.5	14.3	Slight rickets soft bones.
266	+ hydrogenated fat, 10 gram.	21	4,440	6,350 Max. after 10 weeks, 7,300	1,910	20	14	Slight rickets soft bones.

In keeping with the calcium results the bones of the coco-nut oil dogs were hard while those of the hydrogenated fat dogs were soft. This series of experiments illustrates that the anti-rachitic factor has a potent influence on the calcification of the periosteal bone at a time when but slight rickets can be observed at the epiphyses.

X-ray photographs of 264, 265, 266, 267, taken after seventeen weeks of the diet, are seen in Figs. 30, 31, 32, and 33. It will be seen that these radiographic results show the bones to be normal at the epiphyses. There are, however, obvious differences in the shafts of the bones as revealed by the X-rays. In the case of the hydrogenated fat dogs the medullary cavities are broader and the periosteal bone less thick.

COMPARISON BETWEEN COD-LIVER AND OLIVE OILS.

General Diet.

Bread 100 gram. Meat 5 gram.
Salt 1 gram. Separated milk 200 c.c.
Orange juice 5 c.c.
Age of puppies at beginning, seven weeks.

General Diet with Various Oils.

No. of experiment.	Diet.	Duration. weeks.	Initial weight. gram.	Final weight Jan. 26. gram.	Gain. gram.	CaO in femur shaft.		Histology results.
						Dry. %	Fresh. %	
278	+ cod-liver oil, 10 c.c.	7	1,330	1,770	440	26.8	11.2	Normal.
279	+ olive oil, 10 c.c.	7½	1,360	1,770	410	—	8.7	Rickets.

This was a short experiment and was terminated because of an outbreak of distemper in the kennels. The radiographs showed that the animal receiving cod-liver oil (278) remained normal, while rickets was evident in 279 (olive oil).

COMPARISON BETWEEN VARIOUS OILS.

General Diet.

Bread 125 to 175 gram. Meat 10 gram.
Salt 1 gram. Separated milk 175 to 200 c.c.
Orange juice 5 c.c.
Age at beginning of experiment, eight weeks.

No. of experiment.	Diet.	Duration. weeks.	Initial weight. gram.	Final weight. gram.	Gain. gram.	CaO in femur shaft.		Histology results.
						Dry. %	Fresh. %	
282	+ lard, 10 grm. . .	8	2,070	3,800	1,730	20.6	7.76	Slight rickets.
283	+ suet, 10 grm. . .	9	2,170	3,880	1,710	20.0	8.4	Normal.
284	+ bacon fat, 10 grm. . .	8	2,370	3,690	1,320	21.1	8.4	Slight rickets.
			Jan. 27, 4,060 (distemper)					
285	+ pea-nut oil, 10 c.c.	9	1,900	3,630	1,730	21.8	9.9	„
287	+ olive oil, 10 c.c. .	8	1,590	2,600	1,010	—	—	Rickets.
288	+ coco-nut oil, 10 c.c. .	9	2,350	4,320	1,970	—	—	Slight rickets.

282, 283, 284, 285 were retrievers of the same family.

287, 288, were spaniels of the same family.

Both families were of the same age.

These experiments also were short, lasting 8-9 weeks. They were terminated by an outbreak of distemper which necessitated all the dogs being killed. In none of these cases was rickets very marked. Olive oil was the worst and lard also had slight rickets. Of the vegetable oils, coco-nut oil and pea-nut oil gave the best results. The suet dog was quite normal (Fig. 35).

The calcium oxide results show no striking difference throughout the whole series, the lard having the lowest percentage in the retriever set and the olive in the spaniel set. The experiments were too short to allow any significance to be attached to the calcium results, all of which were low because of the early termination of the experiments.

Radiographs of 282, 283, 284, and 285, taken 8-9 weeks after the beginning of the experiment, are shown (Figs. 34, 35, 36, and 37).

COMPARISON BETWEEN VARIOUS FATS.

General Diet.

Bread 100 grm.

Meat 10 grm.

Salt 1 grm.

Separated milk 175 c.c.

Orange juice 5 c.c.

Animals six weeks old at the start of the experiment.

No. of experiment.	General Diet.	Initial Weight. gram.	Weight after 8 weeks 4 days. gram.	Radiographic results after 8½ weeks.
303 (Fig. 40)	+ cod-liver oil . .	1,060	2,600	Normal.
304 („ 41)	+ rape-seed oil . .	1,420	2,970	Slight rickets.
305 („ 42)	+ cotton-seed oil . .	1,070	1,950	Practically normal.
306 („ 43)	+ olive oil . .	1,270	2,190	Rickets.
307 („ 44)	+ lard . .	1,290	2,810	„
308 („ 45)	+ bacon fat . .	980	1,760	Almost normal (7 weeks).

In the radiographs taken after five weeks of diet the olive oil showed the worst rickets, lard the next worst, rape-seed oil slight rickets, the cotton-seed oil was practically normal, and the cod-liver oil was normal (Fig. 40-45). In this series olive oil and lard had the least anti-rachitic action. Except in the case of 303 (cod-liver) and 304 (rape-seed) the experiment ceased at an early age. The radiograph of 304 showed recovery from the slight rickets after twenty-two weeks of the experiment, but the shaft of the fresh femur contained only 9.8 per cent. of CaO, whereas there was 13.6 per cent. CaO in the

THE EFFECT OF BUTTER ON CALCIFICATION.

General Diet.

Type—Cross between small retriever and Airedale.
Age at beginning of experiment, seven weeks three days.

* This high value is undoubtedly due to the absence of fat in the bone marrow as the result of inanition preceding death.

- (1) The presence of meat in the diet even in the early stages of the experiment.
- (2) The animals were living out of doors from birth and throughout the whole period of the experiment.

The addition of meat protein to the diets of 291 and 293 has had little or no effect on the calcification of the periosteal bone but has improved it at the epiphysis (see p. 40).

It may be added that these animals were most severely attacked by mange: in fact, because of the severity of the disease in 291, the experiment was concluded. It was interesting to observe how rapidly

292 and 293 (butter) recovered as compared with 290 and 291 (no butter). No doubt butter, although present in such small quantities, was responsible for the increased resistance to the skin infection.

COMPARISON BETWEEN SUET AND LARD.

In the following experiments the amount of bread eaten also came under close control.

Exps. 328 and 329.

General Diet.

Separated milk 175 c.c. Salt 1 gram.
Bread 100-180 gram. Meat 10 gram.
Orange juice 5 c.c.

Spaniel breed of puppy.

Age at beginning of experiment, seven weeks.

Confined to kennels throughout experiment.

No. of experiment.	Diet.	Duration. weeks.	Initial weight.	Max. weight.	Final weight.	CaO		Radio-graphic results.
						Fresh.	Dry.	
328	G.D. + 10 gram. suet	20	2,210	4,180 Nov. 1	4,180 Nov. 23	12.3	19.2	Normal.
329	G.D. + 10 gram. lard	20	1,870	4,090 Nov. 1	3,490 Nov. 23	7.0	13.7	Very bad rickets.

The radiographs of these two puppies show in marked contrast the rachitic appearance of the bones of 329 (lard), Fig. 39, with the normal condition of 328 (suet), Fig. 38. 329 during the last three weeks was ill and lost weight. Before this illness, however, rickets had developed to a fairly severe degree.

COMPARISON BETWEEN BUTTER AND BUTTER WHICH HAS BEEN HEATED TO 120° C. FOR FOUR HOURS, OXYGEN BEING PASSED THROUGH IT WHILE HEATING.

General Diet.

Separated milk 175 c.c. Salt 1 gram.
Bread 100 gram. Lean meat 5 gram.
Orange juice 5 to 7.5 c.c.

Retrievers. No exercise. Energy of diet kept constant.

Age at beginning, 7½ weeks.

No. of experiment.	General Diet for 8 weeks.	Initial weight.	Weight after 7½ weeks.	Radiographic results after 8 weeks
321	+ 5 to 10 gram. oxidized butter.	2,090	3,850	Rickets.
325	+ 5 to 10 gram. normal butter.	1,800	3,120	Normal.

This experiment was made in consequence of the results obtained by Hopkins (15), who showed that butter heated in this way had lost its Fat-soluble A content. The butter was treated in the above described manner under Professor Hopkins's direction. The radiograph of 321 taken after eight weeks shows evidence of rickets, while

that of 325 is normal (Figs. 46 and 50). After being anaesthetized for this radiograph to be taken, the puppy (321) became ill and never ate its whole diet again. It was killed six weeks later, its weight having fallen from 3,850 gm. to 2,730. The fresh shaft of the femur was found to contain only 5.9 per cent. of CaO, one of the lowest figures obtained in this research. The heated and oxidized butter had apparently lost an element which influences the calcification of bone. Comparative radiographs of 321 (oxidized butter) and 325 (fresh butter) are seen in Figs. 46 and 50.

COMPARISON BETWEEN (1) COD-LIVER OIL, (2) COD-LIVER OIL HEATED TO 120° C. FOR FOUR HOURS, AIR BEING PASSED THROUGH IT DURING THIS TIME, (3) PEA-NUT OIL, (4) OLIVE OIL, (5) COCO-NUT OIL, (6) COTTON-SEED OIL.

General Diet.

Separated milk 175 c.c.

Lean meat 10-15 gm.

Bread 75-180 gm.

Salt 1 gm.

Orange juice 5 c.c.

Retrievers—7 weeks old at start of experiment. Confined to kennels throughout experiment.

No. of experiment.	General Diet.	Initial Weight.	Weight after 10 weeks.	Radiographic result after 10 weeks of diet.
336 (Fig. 52)	+ 10 c.c. cod-liver oil . . .	2,430	5,020	Normal.
337 („ 53)	+ 10 c.c. oxidized cod-liver oil . . .	1,930	4,650	Normal.
338 („ 54)	+ 10 c.c. pea-nut oil . . .	2,310	4,920	Practically normal.
339 („ 55)	+ 10 c.c. olive oil . . .	1,930	4,520	Fairly bad rickets.
340 („ 56)	+ 10 c.c. coco-nut oil . . .	1,620	4,520	Slight rickets.
341 („ 57)	+ 10 c.c. cotton-seed oil . . .	1,800	4,420	Slight rickets.

Radiographic results which agree with the histological findings are shown in Figs. 52-57. The diet was well controlled during the experimental period of ten weeks, as can be seen in the growth curves of these puppies, Fig. 58. Except that the coco-nut oil puppy (340) put on rather more weight than the others, there is good parallelism in rate of growth among them all. The radiographic results are in close agreement with those obtained in experiments of the same type previously described. Olive oil is the worst of these vegetable oils and pea-nut is the best. In view of the destruction of the Fat-soluble A vitamine when butter is heated to 120° and oxidized at the same time, it is surprising that the radiograph of 337, in which case cod-liver oil treated in this way was eaten, shows no indication of rickets or defective calcification. Whether this difference between butter and cod-liver oil can be simply explained by the fact that cod-liver oil contains a much greater quantity of anti-rachitic vitamine than butter, so that the destructive change takes a longer time, or whether some other explanation must be sought, is not known. After ten weeks of the experimental feeding these puppies were anaesthetized for radiographic examination. 339 (olive) died under the anaesthetic, and 341 (cotton-seed), after recovery from the anaesthetic, became ill and gradually lost weight. After fourteen weeks of the diet the

cod-liver and oxidized cod-liver dogs remained normal. Pea-nut oil (338) developed some rickets, but not to the extent of the coco-nut oil puppy (340). The order of potency as regards anti-rachitic effect from this series is again as follows: cod-liver and oxidized cod-liver oil, pea-nut, coco-nut and cotton-seed oils, and lastly olive oil.

SUMMARY.

Certain oils and fats have a great effect in promoting the calcification of bone and preventing the development of rickets, while others have no action in this respect. Of the fats tested, cod-liver oil is the best. Suet and butter also have a potent influence on calcification. Lard is poor as compared with suet. Butter, heated and oxidized for four hours, loses some anti-rachitic action. Cod-liver oil similarly treated still has a strong anti-rachitic effect. The vegetable oils vary in their anti-rachitic action, the order of merit being somewhat as follows: pea-nut and coco-nut oils (best), rape-seed, cotton-seed, palm-kernel, olive, linseed, and babassu oils (worst). Hydrogenated fats are poor.

(b) The question as to whether the Anti-rachitic action of Fats can be explained by their Fat-soluble A vitamine content.

I have now described experimental results which show that fats vary in their influence as regards the calcification of bone and the development of rickets. It is true that there is evidence that bone calcification (in any case periosteal bone calcification) and rickets as indicated by endochondral abnormality are not always identical problems; for in one experiment, the calcium content of the femur shaft was low (9.6 per cent.) (lard), Exp. 181, and there was no rickets, as judged by external appearance and the radiograph, while, in another puppy of the same family (butter), Exp. 182, there was evidence of rickets at the epiphyses when the calcium present in the femur shaft was higher (12.5 per cent.) (for radiographs see Figs. 12 and 13). In Exp. 182 winter butter was eaten, the puppy was confined, it ate much bread and became very fat. It gained 4,660 grms. in 19 weeks, whereas 181 (lard), which ate much less bread, only gained 2,890 grms. in 20 weeks. But otherwise the agreement in results was so general that I shall discuss the anti-rachitic action of fats and their power of promoting calcification as if they were synonymous; for it is probable that the discrepancy mentioned above was due to some other factor of the diet—possibly bread—which in that series of experiments was not controlled.

To explain the variation in the anti-rachitic action of fats on other than a vitamine basis, such, for instance, as the saponification number, the degree of saturation of the constituent fatty acids or the presence of volatile fatty acids, seems hopeless. On the other hand, vitamines in general and, more especially as it concerns this work, Fat-soluble A in particular, are on as firmly established a basis as any other element in the diet, although, it is true, nothing is known of their chemical nature or mode of action. Were there any doubt as to the reality of the Fat-soluble vitamine, greater hesitation in the interpretation of the above-described results would be necessary.

Since there is no doubt on this point, and as there is strong but not complete evidence that the anti-rachitic action of certain fats is bound up with the Fat-soluble element, considered from the point of view of distribution and properties, it can only be assumed that the action of fats in rickets is due to a vitamine or accessory food factor which they contain, probably identical with the Fat-soluble vitamine.

The distribution and properties of the Fat-soluble vitamine have been worked out almost entirely by experiments on the growth of young rats, while, in these experiments showing the effect of fats on rickets, puppies only have been used. Now sufficient is known about variations in the mode of action of the same vitamine in different animals to prevent an investigator being surprised when some discrepancies are evident, and, before a new vitamine is added to the accepted list of three, it is essential that the balance of evidence for and against the identification of the new vitamine with one of the recognized three be carefully considered. This mode of procedure has been adopted in identifying the Water-soluble vitamine with the anti-beri-beri factor and, although some investigators still doubt whether they are the same, the balance of evidence is generally accepted as proving their unity.

Evidence has been obtained in this work which proves that neither the Water-soluble B nor the anti-scorbutic vitamine is responsible for the experimental results obtained in the fat experiments, and I shall proceed to discuss the question of the identity of the Fat-soluble A vitamine with the anti-rachitic substance in fats.

(1) The first point is their distribution. Both are most abundant in the animal fats except lard. Both are relatively deficient in or absent from the vegetable oils. The Fat-soluble vitamine is present in green vegetables but, unfortunately, no successful experiment has been carried through in this investigation proving the presence of the anti-rachitic vitamine in green leaves. The addition of green vegetables or their watery extracts to the diets of puppies gave rise to diarrhoea.

Dealing only with fats, it is clear that the general similarity of distribution of the anti-rachitic and Fat-soluble substances is striking but in detail differences are evident which call for qualification and amplification.

In 1918, when discussing this point in my first publication of this research, I stated that experiments on the distribution of the Fat-soluble vitamine had failed to recognize the presence of this substance in any vegetable oil. My results, however, pointed to the presence of the anti-rachitic vitamine in varying amounts in some of the vegetable oils and that vegetable oils were graded in their action in preventing rickets and promoting calcification of bones. The best of these included pea-nut and coco-nut oils. Linseed, babassu, and hydrogenated fats were the worst oils, while in an intermediate position were found cotton-seed, rape-seed, and palm-kernel oils. At this time I stated that either the Fat-soluble and the anti-rachitic vitamines were different or that the puppy experiments were a more sensitive test as to the presence or absence of the vitamine than the experiments on the growth of rats.

During the past months a part of this difficulty has been removed by the recognition that some of the vegetable oils and lard contain Fat-soluble A. For instance, Daniels and Loughlin (16) have shown that lard and cotton-seed oils are both capable of providing sufficient Fat-soluble vitamine when added to purified synthetic diets devoid of this substance to allow in rats growth, reproduction, and rearing of young. It is true that these fats had to be added in larger quantities (28 per cent. and 21 per cent.) than is usual in such experiments, so that the fact that they contain less of the vitamine than cod-liver oil, suet, and butter is certain, but there seems no longer any doubt that they contain some of the Fat-soluble accessory food factor. Lard contained more than cotton-seed oil. The results are in accordance with my rickets experiences, for lard was on the whole more potently anti-rachitic than cotton-seed oil but not so good as the other animal fats, while cotton-seed oil was better than linseed and olive oils.

Recently also Prof. Hopkins informed me that he had obtained evidence of growth factor in rape-seed oil which is a fat having some of the anti-rachitic vitamine as tested by its action on the production of rickets in puppies. No doubt in the immediate future it will be proved that other vegetable oils contain the Fat-soluble vitamine. I suggest that pea-nut and coco-nut oils are worth investigating from this point of view. It is evident that a part of the discordant facts as to the graded anti-rachitic action of oils and the earlier teaching that lard and the vegetable oils were devoid of the Fat-soluble vitamine is disappearing rapidly since the first publication of my experiments. Obviously much work remains to be done along these lines before complete concordance of results will be obtained.

(2) It has been pointed out above that after a certain age, about four months, it is very difficult to produce rickets in normal puppies by vitamine deficiency in the diet; that is to say, puppies become more independent of the anti-rachitic vitamine from the point of view of bone calcification, especially bone having its origin in cartilage. So also young rats, after obtaining a certain growth, do not stop growing when the Fat-soluble vitamine is excluded from their diet. Rats, in fact, develop a mechanism which makes them less susceptible, from the point of view of growth, to deficiency of this vitamine in the diet. This similarity in behaviour of dogs and rats, in that both develop with increasing age greater independence to vitamine activity as regards such dissimilar functions as calcification of bone and growth, is evidence in favour of the identity of the anti-rachitic and the Fat-soluble vitamins. The question is complicated, for puppies do not develop rickets unless they grow and the more rapidly they put on weight at the expense of substances like bread (see p. 42), the more severe will be the rickets. In the case of young rats, however high the energy of the food eaten, they will not continue to grow unless there is a supply of the Fat-soluble vitamine. Puppies, on the other hand, grow well when there is only a minimum of Fat-soluble A present in the food. It is unlikely that the food of the puppies which developed rickets was entirely devoid of this vitamine, but it can be safely stated that it was only present in very small amounts, and

that the growth of the puppy had no relation to the amount eaten. If, at the end of this period, bad rickets had developed, then the animal often went off its food and its weight declined. Generally a puppy receiving linseed oil would grow during the early months as well as one getting cod-liver oil if they ate equivalent amounts of other foodstuffs. The weight curves seen in Fig. 58, where the diets were closely controlled and everything was constant except the type of fat eaten, are good evidence of the independence of growth and the amount of Fat-soluble A eaten, for it will be seen how parallel are the rates of growth of these puppies. Since 175 c.c. of separated milk was one of the elements in the diet, it is certain that some Fat-soluble vitamine was eaten, but, in the case of animals of 4,000 grm. weight, this amount must have been exceedingly small. It would be interesting to carry out experiments in which puppies were fed on synthetic diets of the purified elements similar to those used in the rat feeding work. If puppies could be got to eat a diet of this nature, it would settle the question as to whether the Fat-soluble vitamine is as essential for growth in the case of puppies as it is in rats. I am doubtful whether, in applying vitamine results to children, too much is not being made of their effect on growth. This opinion depends not only on the results of feeding puppies on vitamine-deficient diets but also on the observations on children made by Hess and Unger (17). These workers fed children on separated milk powder, sucrose, autolysed yeast, cereal and cotton-seed oil, under the impression that all these substances were devoid of Fat-soluble A. Even over a period of fifteen months' feeding on this diet, the children grew, although at a less rate than normal. Hess and Unger decided that Fat-soluble A cannot be a factor of practical importance in the growth of children because it is unlikely that the diet of a child would ever contain as small a quantity of this vitamine as in the above-described diet. With their further conclusion that Fat-soluble A or a vitamine with a similar distribution which I have called the anti-rachitic vitamine, has but little to do with rickets in children, I do not agree. I have referred to this work in an earlier publication (2), and will discuss it again in a later paper.

The question arises as to whether any suggestion or explanation of the different susceptibility of the young rat and puppy as regards growth to the Fat-soluble factor of the diet can be made. It may be a question of age and length of time of reaction. Probably the puppy develops some mechanism making it more independent of the anti-rachitic factor before it is 5-8 weeks old. Certain it is that the younger the puppy the greater is the difficulty in getting growth and retaining health on these diets deficient in Fat-soluble vitamine.

It is evident that the question of the relative importance of Fat-soluble A in the growth of rats as compared with the growth of puppies and children cannot be settled without further experiment, but there is good evidence that it does not hold the important position in the latter animals that is generally assumed by those engaged in the rat-feeding experiments.

Besides the similarity in reaction between the growth of rats

and the production of rickets in puppies, in which cases there develops in the animals a greater independence to the Fat-soluble and anti-rachitic vitamine respectively, there is one other physiological point of agreement in reaction which appears suggestive. When young healthy rats are suddenly placed on a synthetic diet devoid of Fat-soluble A, the effect of the deficiency is not noticed at once. The rats usually continue to grow at a normal rate for about twenty days. The effect is by no means immediate. Similarly in the case of feeding on diets deficient in anti-rachitic vitamine, the effect is slow and even under the best rickets-producing conditions there is usually but little result under six weeks of treatment. The calcium metabolism and balance seem to be very little influenced, as evidenced by the calcium content of the bones, until the puppy has eaten the diet deficient in anti-rachitic factor for some weeks. When, however, we consider either rats or puppies whose diets have been deficient in Fat-soluble vitamine so that, in the one case, growth has ceased or, in the other, rickets has developed, then the addition of a fat containing the vitamine immediately produces its effect by stimulating growth and by improving the calcification of bone in the respective cases.

These facts of agreement from the point of view of physiological reaction seem to me strong evidence that the substance in fats stimulating the calcification of bone is the same as Fat-soluble A, i. e. the factor which stimulates growth in rats. Their delayed action in the healthy animal also suggests that both effects, stimulation of growth and calcification of bone, are indirect and not direct. The greater independence of older animals indicates the same type of action.

(3) The third point as to the identity of the Fat-soluble and anti-rachitic vitamines involves a consideration of the properties of these bodies, apart from their distribution and mode of action. It is necessary to add that, even in the case of the Fat-soluble vitamine, we know very little and our knowledge as to the properties of the anti-rachitic vitamine is much more scanty.

Effect of Heat. There is some discordance of results as to the susceptibility of Fat-soluble A to heat but the work of Osborne and Mendel (18), who showed that butter treated with steam for $2\frac{1}{2}$ hours still retained its growth-promoting qualities, is generally accepted as correct. In other words, Fat-soluble A is strongly resistant to heat when present in fats. In the case of cod-liver oil there is strong heat resistance so far as the anti-rachitic action is concerned. Experiments are quoted above (229, 231, and 232) (p. 24) where cod-liver oil was heated to 120°C . for periods of 2 and 4 hours. There was no evidence of destruction of the anti-rachitic vitamine after two hours' heating, but in the case of four hours' heating there may have been some slight destruction as, at one period of the experiment, the radiograph of this animal was not quite normal. The abnormality, however, was very small and may have been due to an uncontrolled factor, viz. the amount of bread eaten. It was quite clear from this experiment that the anti-rachitic vitamine like the Fat-soluble vitamine is strongly thermo-stable.

The effect of oxidation and heat. Part of the discrepancy in the

experimental results as to the effect of heat on Fat-soluble A is no doubt due, as pointed out by Hopkins (15), to the possibility of oxidative changes being allowed to proceed side by side with the heating process. For he has shown that, if oxygen is allowed to pass through heated butter, the Fat-soluble vitamine is readily destroyed. Rickets experiments were carried out to see if there was much anti-rachitic vitamine left in butter heated to 120° for four hours with oxygen passing through it continuously. This process was kindly carried out in Prof. Hopkins's laboratory. It was found that animals fed with this fat had extremely little calcium in their bones as compared with animals similarly fed but eating the untreated butter. There was no doubt but that the anti-rachitic vitamine had been to some extent destroyed when butter was treated in this way (see Exp. 321-5) (see p. 29).

In order to emphasize more strongly the difference between a normal oil and the same after subjection to heat and oxygen, I tried the effect of cod-liver oil which had been heated to 120° C. for four hours while oxygen was passed through it. Both the animals, the one receiving the untreated oil and the one receiving the oxidized oil, remained normal (Exp. 336 and 337), and there was no evidence of the destruction of the anti-rachitic vitamine. If it should happen that four hours' heating and oxidation at 120° C. also leaves a large amount of Fat-soluble A in the cod-liver oil, it will go a long way, especially when considered together with the butter results, to clinch completely the identity of Fat-soluble A and the anti-rachitic vitamine.

I have now discussed the question whether the anti-rachitic action of certain fats is due to their Fat-soluble A content. It is clear that there is strong evidence in support of the contention that the promotion of growth of young rats and the increased calcification of bones of puppies are due to the same vitamine in fats. Some points still await investigation and, until this work has been done, it cannot be confidently asserted that they are identical substances. It is important that further attempts should be made by experiments on the growth of rats to see if more data as to the distribution of Fat-soluble A in vegetable oils can be obtained in accordance with the anti-rachitic action of fats as explained above. Another problem urgently requiring solution is an investigation into the anti-rachitic action of green vegetables.

The interaction of the anti-rachitic vitamine with the other elements of the diet and environment, some of which work hand in hand with the vitamine, while others antagonize it and have a rickets-producing effect, will be dealt with in other sections of this publication.

SUMMARY.

(1) The evidence is discussed as to whether the action of fats in the development of rickets is due to a vitamine, and whether this vitamine is the same as Fat-soluble A. The distribution of the substance in fats having the anti-rachitic action and the few properties as regards heat and oxidation which have been investigated, lend strong support to its identification with Fat-soluble A.

(2) The time relations of the reactions in growth and rickets

experiments produced by the various fats are similar. In both cases, when vitamine-deficient but otherwise adequate diets are given to well-fed healthy rats and puppies, there is a long interval before their effect on growth and calcification respectively is obvious. When the animals are suffering from the deficiency the addition of a good fat to the diet produces its effect quickly.

Rats as regards growth and puppies as regards rickets become more independent of the vitamine as they grow older. These facts are in favour of Fat-soluble A being also responsible for the anti-rachitic action of certain fats.

(c) The Influence of Meat and its Constituents.

Meat has long had the reputation in therapeutics of assisting in the curative treatment of rickets in children. So far as I know, the knowledge was purely empirical, and it was one of the recommendations among others, such as the addition of milk and cod-liver oil to the diet, made in the treatment of rickets. The fact that fresh scraped meat is given in this disease suggests that, when introduced as a therapeutic agent, infantile scurvy may have been either the disease treated or a complication of the pathological condition.

In the case of puppies, the addition of meat to diets devoid of this substance reacts favourably on their health. It is the first substance to be picked out of a food mixture, and it is evident that its special position of favour is justified by the important part it plays in nutrition. The addition of 10 grm. of meat a day to the standard diet will often transform a puppy, off its food and losing weight, into a vigorous animal, eating its full ration and putting on weight. So striking is this effect that, in most of the later experiments where it has been necessary to make each puppy of a series eat its diet quantitatively, meat has formed an element of the food. It might be expected that meat would have an anti-rachitic action. That this is the case I shall now show. After demonstrating its action in inhibiting the development of rickets, I shall discuss the limitation of its anti-rachitic effect. In all the experiments described below the fresh meat given had the fat dissected off as far as was possible.

COMPARATIVE EFFECT OF ADDING 10 AND 50 GRM. OF MEAT TO A RICKETS-PRODUCING DIET.

General Diet :

Separated milk 175-250 c.c. Orange juice 3 c.c.
White bread. Linseed oil 10 c.c.
Yeast 5 grm. Salt 1 grm.

Age at beginning 7 weeks.

No. of experi- ment.	General Diet.	Duration. weeks.	Initial weight. grm.	Final weight. grm.	Gain. grm.	CaO in femur. Fresh. %	Histology results.
174	+ 10 grm. lean meat.	22	1,800	5,115	3,315	8.7	Rickets.
175	+ 50 grm. meat .	22	2,040	6,625	4,585	9.04	Rickets (less than 174).
172	+ 10 grm. butter, + 10 grm. meat.	22	1,600	5,575	3,975	13	Nearly normal.

Radiographs of 174 and 175 taken after thirteen weeks of the experiment are shown in Figs. 60 and 61. Whereas both animals developed rickets, 174 receiving only 10 gm. of meat per diem was slightly worse, although it grew at the slower rate. It will be noted that the bones in both animals had a low calcium content, but the radiographs and histological sections show that rather more severe rickets was present in 174. This is one of the characteristic effects of meat. Its action on endochondral calcification is more easily observed than on the calcium deposition in periosteal bone. In some cases the radiographic appearance obtained in animals eating meat together with a rickets-producing diet may appear more normal than the histological picture and calcium content of bone would lead to expect. There was no puppy in this series not eating some meat, so that these results cannot be contrasted with a control of the same litter. Reference, however, to other puppies of the research on the same diet as above, not eating meat, will show that the rickets of 174 (10 gm. of meat) was not so strongly developed as it would otherwise have been—compare Fig. 60 with Figs. 7 and 95. Fig. 59 is a radiograph of a puppy of the same series as 174 and 175 which received an additional 10 gm. of butter. This has protected the animal against rickets better than the extra 40 gm. of meat eaten by 175.

COMPARATIVE EFFECT OF DIETS WITH AND WITHOUT MEAT.

General Diet :

Separated milk 175 c.c. Orange juice 5 c.c.
White bread 50–180 gm. Linseed oil 10 c.c.

Puppies probably not of same family but of same age.

<i>No. of experi- ment.</i>	<i>General Diet.</i>	<i>Other Conditions.</i>	<i>Dura- tion. weeks.</i>	<i>Initial Weight. gm.</i>	<i>Weight after 8 weeks. gm.</i>	<i>Gain. gm.</i>	<i>Radio- graphic results.</i>	<i>Histology results.</i>
351	+ 20–30 gm. lean meat	Liberty during day.	16	1,380	2,660	1 280	Very slight rickets.	Some rickets.
352	—	Liberty during day	16	1,370	2,960	1,590	Rickets.	Rickets.
353	+ 20–30 gm. lean meat	Confined to kennel	16	1,730	3,930	2,200	Slight rickets.	Rickets.
354	—	Confined to kennel.	16	1,760	3,730	1,970	Rickets.	Bad rickets.

In this experiment all articles of diet were eaten quantitatively. The kennels were in the open air throughout the experiment. From the point of view of the effect of meat, 351 and 352 are comparable, similarly 353 and 354. The radiographs of these puppies taken 8 weeks after beginning experiment are seen in Figs. 62–65. Very slight rickets is present in either of the animals eating meat, 351 and 353, but 352 and 354 (no meat) have both developed fairly advanced rickets. The contrast in the epiphyseal swellings at the wrist-joints was interesting, both the meat-eating animals remaining more nearly normal while 352 and 354 (no meat) developed the characteristic swellings. This series of experiments will be referred to again in the

section dealing with exercise. Growth curves of these puppies are shown in Fig. 66.

Although it is evident from the above experiment that the anti-rachitic effect of meat is a real thing, more particularly as regards the epiphyses of bones, very bad rickets can be produced in large rapidly growing dogs, eating a lot of bread, even when as much as 50 grm. of fresh lean meat are added to the diet. The following experiments illustrate this point.

General Diet :

Separated milk 175 c.c. Bread *ad lib.*
Yeast 5-10 grm. Linseed oil 10 c.c.

Retrievers. Age at beginning 7 to 8 weeks.

No. of experi- ment.	General Diet.	Dura- tion. weeks.	Initial weight. grm.	Final weight. grm.	Gain. grm.	CaO in femur shaft		Remarks.
						Fresh. %	Dry. %	
141	+ 5 grm. meat.	20	2,490	5,820	2,330	7.19	17.48	Rickets
143	+ 20 grm. meat	20	2,890	4,400	1,510	9.48	17.88	Rickets.
144	+ 50 grm. meat	20	3,690	8,825	5,735	10.72	15.74	Ill towards end of experiment. Very bad rickets.

Rickets developed in all these puppies, 144 being very bad in spite of receiving most meat. Fig. 51 is a radiograph taken some time after death, and is therefore rather blurred, but still conveys the impression of the intensity of the disease. This animal gained weight very rapidly, owing to the large amount of bread eaten in addition to the meat. Since increasing the bread in a diet, other things being equal, increases the rickets produced (see section on bread), and since the addition of meat creates the desire to eat more bread, it is clear that the anti-rachitic effect of meat may be more than counterbalanced, especially in a large breed of dog, by the increased amount of bread eaten. This has happened in Exp. 144 (Fig. 51). It is of interest to note that, although 144 had apparently the worst rickets in this series, its periosteal bone contained the highest percentage of calcium.

The question now arises to what the anti-rachitic action of meat is due. It may be stated at once that this problem has not been solved. The action seems to be of an altogether different order from that of the fats containing the anti-rachitic vitamine. It may be suggested that it is due to the small amount of vitamine associated with fat which cannot be removed from the meat by dissection. This is a possible, but, in my opinion, an unlikely explanation of its whole action. In an earlier publication I suggested that its beneficial effect was possibly due to its stimulant action on the metabolism (specific dynamic action), which in the case of meat is high. It can readily be imagined that anything which stimulates metabolism will bring into greater action any anti-rachitic vitamine either in the food or in the animal's body. And on this basis the effect of meat as an adjuvant to the anti-rachitic action of other physiological substances might be placed. This explanation would put the anti-rachitic action of meat and that of exercise on a similar basis, and I think there

is something in common between the two since both appear to be of a secondary nature to the anti-rachitic vitamine. If, however, the stimulation to metabolism alone were the real explanation of the action of meat in rickets, it would be expected that any method of increasing metabolism would produce an anti-rachitic effect. For instance, thyroid extract might be expected to assist in preventing rickets. Recent experiments performed to decide this point have not led to the expected result, for in spite of large daily doses of thyroid gland, rickets developed almost as severely as in the control animal receiving no thyroid. In the experiments quoted below (p. 69) there was no doubt that the thyroid in the diet stimulated the metabolism, because, although both animals ate food containing the same constituents and same amount of energy, the puppy receiving thyroid put on weight much less rapidly. In view of this result with thyroid, I am doubtful whether the anti-rachitic effect of lean meat can be satisfactorily explained by its specific dynamic action and the increased metabolism it produces. The action of meat in preventing rickets is also probably closely related to its effect in improving the health and general disposition of young animals, a fact previously alluded to, and the solution of the latter problem would also no doubt explain its anti-rachitic effect.

Finally, there is always the possibility that the anti-rachitic effect of meat, under some conditions, may be due to its power of promoting the digestion and absorption of other foodstuffs from the alimentary canal.

A few experiments have been made to see whether the action of meat in inhibiting rickets is due to its protein or to its extractive content. The problem is especially difficult because the anti-rachitic action of meat *per se* is small, and it is therefore necessary to control closely all the other elements of the diet in addition to the environment.

In the following experiments (299 and 301), in which everything was closely controlled, it will be seen that meat protein has some anti-rachitic action, more particularly obvious at the points of endochondral ossification, but once again, as in the case of meat, with no effect on the calcification of the periosteal bone.

Exp. 299 and 301 :

General Diet :

Separated milk 200 c.c.	Salt 2 gram.
Bread 70-175 gram.	Linseed oil 10 c.c.
Orange juice 5 c.c.	Meat 10-20 gram.

Age at beginning about 6 weeks.

No. of experiment.	General Diet.	Duration. weeks.	Initial weight. gram.	Final weight. gram.	Gain. gram.	CaO		Radiographic result.	Histology result.
						<i>in femur</i> Fresh bone.	<i>shaft.</i> Dry bone.		
299	+ 20 gram. meat protein	18½	2,340	6,650	4,310	% 9	% 12.8	Almost normal.	Rickets.
301	+ 30 gram. bread	18½	1,800	6,560	4,760	9.25	13.3	Rickets.	Bad rickets.

The energy of the diets of these dogs was kept as constant as possible, that is to say, the meat protein of 299 replaced bread; 30 grm. of bread being regarded as equivalent to 20 grm. of meat protein. Both animals at the end of the experimental period looked rachitic. The radiographs show 301, Figs. 69 and 72 (no meat protein) to have developed the condition to a more severe degree than 299, Figs. 67 and 70. Histological evidence of the bones shows 299 to have obvious rickets but not as bad as 301. It is clear then that the substitution of bread by meat protein has slightly reduced the rickets at the epiphyseal ends. It will be further seen, however, that the long bones of 299 are by no means normal, and, in fact, contain slightly less calcium than those of 301, which radiographically and histologically has worse rickets. Both calcium results are below the normal. For other experiments on meat proteins see Exps. 290, 291, 292, and 293, p. 28. Thus we see that the action of meat protein is very similar to that of lean meat itself. In Exp. 299, 20 grm. of meat protein were added to the diet. This is equivalent to 100 grm. of meat, a large ration for a puppy in this research, and, as compared with the amount used in the meat experiments, a quantity which might be expected to produce a greater anti-rachitic effect than that observed. It seemed probable, in fact, that the extractives of meat were responsible for some of the inhibitory action on rickets development possessed by meat.

Several series of experiments have been made to test the action of meat extract in rickets. The earlier results pointed to a definite anti-rachitic effect, but later work, when other factors were more closely controlled, suggested that this action was smaller than previously indicated. Before making a definite pronouncement on the extent of the anti-rachitic effect of meat extracts, I wish to repeat the earlier work under more precise conditions. In the meantime it is safe to say that the effect of meat extractives is less than that of meat itself.

The effect of meat on the development of rickets will be considered later in relation to exercise and confinement.

SUMMARY.

(1) Lean meat has a definite anti-rachitic effect. Its action in this respect is, however, of a secondary nature and is probably more prominent in assisting any anti-rachitic vitamine there may be present in the diet. When freedom is allowed to the puppy, meat increases its activity (see p. 64).

(2) Its anti-rachitic action may to a slight degree be due to anti-rachitic vitamine contained in the fat inseparable from it by dissection, but this does not explain the whole effect. The health of the animal is greatly benefited by the addition of small quantities of meat to the diet, and the basis of this improvement in health, whatever it may be, probably also affords the explanation of its inhibitory effect on rickets development.

(3) It appears to assist calcification processes at the epiphyses more prominently than the calcification of periosteal bone, so that the

radiographs may appear nearly normal when the calcium content of the bone shafts is subnormal.

(4) The protein of meat has the same kind of inhibitory action as meat itself, but to a less degree. A definite pronouncement as regards the anti-rachitic action of meat extractives which, in any case, is small, must wait until further experiments are completed under more precise conditions.

(d) The Influence of Bread and Carbohydrates.

Many clinicians, Cheadle (19), have thought that excessive carbohydrate plays a part in the production of rickets, and I now propose to give experimental proof that this is the case.

Although bread has formed an element of the diets employed to produce rickets in puppies since the early days of this work, it was a long time before it was clearly established that, not only had bread no anti-rachitic action, but that it was a most important substance in actually making the condition worse. All investigations are influenced by the results obtained in allied experimental work, and there was practically no indication from the beri-beri and scurvy researches that the presence of any element in the diet could be considered as having a disease-producing effect. These deficiency diseases were (and are) usually held to be the result pure and simple of deficiency respectively in the anti-beri-beri and anti-scorbutic factor. The results to be described will make it clear that rickets cannot be classed as a 'deficiency disease' in this sense of the word.

In my first publication on rickets I emphasized the close relation between rickets and growth. Not only is growth essential before rickets develops but also the greater the growth (in this case the greater the increase in weight¹) the worse is the condition of rickets produced. Only slowly did the idea crystallize that excess of² bread might be responsible for the exaggeration of the rachitic syndrome.

The earliest experiments, where the action of bread became apparent, have already been described (p. 24). Three puppies (220, 221, and 223, Figs. 15, 21, 18) were on similar diets containing palm-kernel oil, and all the elements of the diets were carefully measured except the bread, which was given according to the dogs' appetites. 220, which ate most bread and put on weight very rapidly, developed very bad rickets, while in the same period 221 had only slight rickets. Intermediate from the point of view of rickets was 223, which put on weight at a pace intermediate between 220 and 221. Unfortunately in this series of experiments no tally was made of the actual amount of bread eaten. In repeating the experiment on other puppies, a record of the amount of bread eaten was obtained. All the other elements of the diet, except bread, remained constant.

Puppies 319 and 320 ate

¹ For the purpose of this paper I am using these words as synonymous and this use is not strictly accurate.

² It will be clear from the work described in this paper that 'excess' and 'deficiency' of a dietetic element can only be considered as such after reference to the other elements of the diet.

General Diet.

Separated milk 200 c.c. Salt 1 gram.
 Orange juice 5 c.c. Meat (10 gram. first 4 weeks).
 Linseed oil 10 c.c.

In addition there was given

319	320
100-250 gram. bread.	75 gram. bread.

Type of puppies, Black Lurchers.

Age at beginning, eight weeks.

Animals confined to kennels throughout.

In eight weeks 319 (large amount of bread) increased in weight 1,560 gram. In the same period 320 (small amount of bread) increased in weight 840 gram. In this period 319 (Fig. 74) had developed advanced rickets, whereas 320 (Fig. 73) was much more normal.

As often happened in these experiments, after severe rickets had developed, 319 refused to eat its diet completely, and its weight declined from 5,100 gram. to 4,850 in the last fifty-three days of its life. In this same period 320 (less bread) increased in weight from 3,330 to 3,650 gram. (Fig. 75). At death the following percentages of CaO were found in the shafts of the femurs of these dogs.

No. of experiment.	Percentage of CaO in		Histology results.
	fresh femur.	dry femur.	
319 (Fig. 74) . . .	9.1	18.3	Bad rickets.
320 (Fig. 73) . . .	11.2	18.5	Rickets.

The calcium content of the fresh bones, which, as previously explained (p. 10), is the important number, shows that 319 had developed worse rickets as the result of eating a greater quantity of bread. The minute anatomy of the bones agreed with the radiographic and chemical results. See Figs. 118 and 119.

In the following series also the amount of bread eaten was the only variable in the diet.

Puppies 333, 334, and 335 ate

General Diet.

Separated milk 175 c.c. Orange juice 5 c.c.
 Meat 5-20 gram. Salt 1 gram.
 Linseed oil 10 c.c.

Type of puppies—terriers.

Age at beginning, eight weeks.

Confined (indoors) to kennels throughout.

They received in addition

333	334	335
50 gram. bread.	100 gram. bread	150-180 gram. bread.

The weight curves of these puppies is shown in Fig. 79. It will be seen that in a period of ten weeks :

333	increased from 1,500 to 2,470—	970 gram. gain.
334	“ “ 1,600 to 3,330—1,730	“ “
335	“ “ 2,100 to 5,090—2,990	“ “

The radiographs show that 335 (Fig. 78) has very bad rickets, 333 (Fig. 76) slight rickets, while 334 (Fig. 77) is intermediate between these two.

No. of experiment.	Percentage CaO in femur.		Histology
	Fresh shaft.	Dry shaft.	
333 (Fig. 76) . .	10.95	17.5	Rickets (Fig. 126).
334 (Fig. 77) . .	8.92	14.7	Rickets.
335 (Fig. 78) . .	8.32	13.9	Bad rickets (Fig. 127).

The calcium content of the shafts of the bones are in keeping with the radiographic results, the animal with the worst endochondral calcification having also the smallest percentage of CaO in the periosteal bone.

In this experiment also the dog that developed the worst rickets as the result of eating most bread became ill during the latter weeks and declined in weight from 5,090 to 4,510 gm. in the course of the last three or four weeks. Neither 333 nor 334, eating smaller quantities of bread, suffered in this way, but they continued to eat their full ration and gained weight slowly.

In view of the above results no doubt can remain but that, when eating diets capable of producing rickets, the severity of the disease depends upon the amount of bread eaten, the larger the amount consumed the more severe is the disease produced, when all other factors are kept constant.

Although experiments have not been made to determine the point directly, it is probable that other cereals would act in a similar way to bread, for in earlier experiments it has been shown that oatmeal and polished rice in the diet of puppies are compatible with the development of rickets. I think it probable, however, that differences will be shown among the cereals. I should expect that oatmeal would not be so rickets-producing as white wheaten flour, nor unpolished as bad as polished rice. It may be added that oatmeal contains five times as much CaO as white wheaten flour and natural rice thirteen times as much as polished rice.

In view of the above results I attempted to find out whether any particular constituent of the bread was more important than others in producing rickets. In this quest some success was obtained, for there is evidence that pure carbohydrate is capable of producing bones that are more defectively calcified. Several attempts were made to test this point, but the great difficulty met with was that the puppies became ill when given diets deficient in the anti-rachitic vitamine and containing, at the same time, excess of pure carbohydrate. To establish a fact of this nature it is essential that the puppies should eat the diets quantitatively. Resort had finally to be made to the replacement of linseed oil, which is one of those oils containing the least anti-rachitic action, by lard, which is rather more antagonistic to rickets and is compatible with better health under the conditions of the experiments. The meat ration was also increased to 20 gm.

In the following series of experiments severe rickets was not produced in any case, and the best indication of the detrimental action

of the additional carbohydrate (glucose) can be seen in the calcium results.

Experiments 295, 296, and 297 received as a

General Diet.

Separated milk 200 c.c. Orange juice 5 c.c.
Bread 100 grm. Lard 10 grm.
Meat 20 grm.

Eight weeks old at beginning.

<i>No. of experiment.</i>	<i>Diet</i>	<i>Dura- tion. weeks.</i>	<i>Initial weight. grm.</i>	<i>Final weight. grm.</i>	<i>CaO</i>	
					<i>Fresh. %</i>	<i>Dry. %</i>
295	+ 50-90 grm. of glucose per diem	19	2,410	6,330	9.6	12.7
296	+ 10-30 grm. of glucose per diem	19	1,870	4,080	11.6	15.9
297	General Diet only	19	1,810	4,460	14.0	18.8

The radiographs show that 295 (most glucose) was most rachitic but, even in this case, the degree of rickets as indicated by defective endochondral calcification was not great. 297 (no glucose) was almost normal. The chemical results also show that the more carbohydrate eaten the greater the defect in bone calcification, when all other factors of diet and environment are constant. The histological results are in accord with the chemical determinations.

It is probable, then, that the carbohydrate moiety of bread is largely responsible for the fact that increasing the intake of this substance relatively to the rest of the diet makes the severity of the disease worse. Bread may contain other offending constituents, but this problem has not been worked out.

How the action of bread in producing rickets is to be explained can only be a matter of conjecture at this stage. Like meat it is deficient in both Fat-soluble A and calcium salts, but as regards rickets its action is opposite in nature to that of meat. Whereas meat tends to diminish or prevent the increase of epiphyseal swellings, bread encourages their hypertrophy. Meat increases endochondral ossification, bread inhibits it. It is true that the greater the amount of bread eaten the greater is the increase in weight and formation of new tissues, and this in itself must result in a more urgent call on the calcium in the body, so that less is available for the calcification of bone. But the same effects are produced by eating more meat, which does not have the rickets-producing action. However, the comparison cannot be pressed because in the bread-variable experiments, bread amounting to 100-250 grm. was eaten each day, whereas in the meat-variable experiments the meat did not exceed 50 grm. per diem. With amounts of meat, comparable to those used in the bread experiments, it might be found that this foodstuff also increased the severity of rickets. Certainly very bad defects in calcification of bone can be produced when rapidly growing puppies eat fair quantities of meat (see Exp. 144) if the diets are otherwise unbalanced and especially when excess of bread is eaten.

The detrimental action of excessive bread on the health of the puppies is a most striking fact. It must be remembered that the

diets in these cases were also deficient in Fat-soluble A and the animals were not being exercised. But the bad results of feeding were certainly due to the bread, for when this was reduced or kept low these animals remained comparatively healthy. The large amount of bread produced, in the first place, slowness of movement, then lethargy, and in some cases, paresis, especially of the hind legs. Ultimately the puppies refused to eat up their food, lost weight, and looked generally miserable.

SUMMARY.

(1) Increasing the bread in diets deficient in the anti-rachitic vitamine, has a great influence in increasing the severity and rate of development of rickets, especially in animals receiving no exercise: This statement probably holds also for other cereals, but the point has not been proved.

(2) In one series of experiments it was shown that glucose also produced more defective calcification of bone, so that it is possible that the carbohydrate moiety of bread is largely responsible for this detrimental action.

(3) Under the conditions of the above described experiments, excessive bread, besides its inhibitory action on calcification, had a very bad effect after six to ten weeks on the general health of the animals, which ultimately became lethargic, refused food, and lost weight. In view of the large part bread plays in the dietary of the people, the above facts cannot be too strongly emphasized.

(e) **The balanced action of Milk constituents.**

In earlier sections of this publication it has been seen that dietetic elements have two actions, the one stimulating calcification processes and preventing rickets, the other inhibiting these processes and tending to produce rickets. The result depends on the nature of the balance of these actions. This generalization is crude because it expresses neither the close interplay between all the dietetic elements among themselves nor the dependence of their actions on the environment nor the fact that rickets is something more than defective calcification. In this chapter I wish to describe some preliminary experiments made to elucidate the action of milk constituents in rickets and to show the importance of the balanced effect produced by these elements as they are normally found in this food. It will be seen that, when the constituents of milk are altered in relation to each other, detrimental developments may occur in the animal ingesting them.

(i) 'CASEIN'.

Meat has been shown to have a definite action in aiding calcification of the endochondral bone, although but slight effect on that of the periosteal bone, and the same type of action, but to a smaller degree, was produced by meat protein. It might be surmised therefore that casein would act similarly. In the earlier experiments performed, it was found that, contrary to expectations, casein had but little action on the development of rickets. In later experiments, however, the casein preparation used hastened the onset of rickets—

or a condition almost identical with it so far as the external appearance of the animal and radiographic examination are concerned—to an advanced degree. Closer investigation of the problem made it apparent that the effect obtained depended on the type of casein employed. In the earlier experiments 'edible casein' was added to the diets. This preparation was alkaline and went into solution more readily. The substance used later was caseinogen. It was acidic in reaction and no doubt prepared from milk by acid precipitation. Both preparations were commercial. I shall describe the results obtained with these substances separately.

Edible Casein (alkaline in reaction).

Exp. 300 and 301.

General Diet.

Separated milk 200 c.c.	Orange juice 5 c.c.
Bread 70–175 grm.	Meat 10–20 grm.
Linseed oil 10 c.c.	Salt 2 grm.

Retrievers.

Age at beginning about 6 weeks.

Confined to kennel throughout.

No. of experiment.	Diet.	Dura- tion. weeks.	Initial weight. grm.	Final weight. grm.	Max. weight. grm. (after 15 weeks.)	CaO in		Histology results.
						Fresh bone. %	Dry bone. %	
300	+ 20 grm. casein	18½	2,150	5,010	5,420	7.7	18.4 (no fat in marrow in bone).	Rickets.
301	+ 30 grm. bread	18½	1,800	6,560	6,560	9.25	13.3	Bad rickets.

Energy of food eaten was practically constant for first 15 weeks.

After 15 weeks of the experiment, 300 (casein) went off its food and its weight decreased from 5,420 to 5,010 grms. The relatively high calcium content in percentage of the dry bone in 300 is due to the absence of fat in the bone marrow which disappears early in illness. When the experiment had continued 13 weeks, the radiographs (Figs. 68 and 69) of these animals both showed some rickets of equal degree. At death, however, after 18½ weeks of feeding, 301 (Fig. 72) had more advanced rickets, while in 300 (Figs. 68 and 71) (casein) curative changes had started. The interpretation of the experiment is, possibly, that casein had but little influence in inhibiting the development of rickets but that, when the puppy became ill and refused to eat its diet, especially the bread, completely, the rate of growth decreased and curative processes were set up at the epiphyseal ends.

It will be noticed that the casein dog (300) at death had a rather less percentage of calcium in its bones than 301 (7.7 per cent. CaO in fresh femur shaft as compared with 9.25 per cent. in control animal). In the section of this paper dealing with the effect of meat protein, an experiment of this series (299) was described wherein it was shown that meat protein improved endochondral calcification but had less effect on the periosteal calcification. These experiments show that edible casein, when substituted for an amount of bread equivalent as regards energy, has but little effect on rickets.

Caseinogen (acidic in reaction). I shall now show that an acidic sample of casein (probably ordinary caseinogen made by acid precipitation of milk) has very definite rickets-producing action (see Exps. 321-5). I use the word 'rickets-producing' in this case because the condition as regards external appearance and radiographic examination was indistinguishable from rickets. There was some osteoporosis as well as rickets. I wish to emphasize that all these animals received an equal and an adequate amount of calcium in the separated milk of the diet so that any osteoporotic condition that may have developed was not equivalent to the state produced by Miwa and Stoeltzner (20) when they fed puppies on diets deficient in calcium. The histological examination of this material shows that the rachitic changes were attended with osteoporosis.

The object of this series of experiments made on five retrievers of the same litter was twofold in the first place.

(1) To test the effect of casein on rickets development and to see whether the removal of any Fat-soluble A from commercial samples of 'casein' by prolonged extraction with alcohol made a difference to its effect on the production of rachitic changes.

(2) To see whether heating butter 120° C. for 4 hours and passing oxygen through it during this period and so destroying its Fat-soluble A content would destroy its anti-rachitic action.

The second of these points has already been discussed (p. 29), where it was shown that destroying the Fat-soluble A content of butter had a definite effect in inhibiting the calcification of bones of the animals.

General Diet.

Separated milk 175 c.c.

Salt 1 gm.

Bread 100 gm. +

Lean meat 5 gm.

Orange juice 5-7.5 c.c.

In addition the animals received for 8 weeks the following substances :

321. 5-10 gm. autoclaved and oxidized butter (4 hours).

322. 5-10 gm. fresh butter + 10-20 gm. 'casein' (unextracted).

323. 5-10 gm. autoclaved and oxidized butter + 10-20 gm. 'casein' (extracted).

324. 5-10 gm. fresh butter + 10-20 gm. 'casein' (extracted).

325. 5-10 gm. fresh butter.

After eight weeks of the above diet, the radiographs show the following changes (Figs. 46, 47, 48, 49, and 50).

321—rickets, 322—rickets, 323—very bad rickets, 324—rickets, 325 normal.

The deductions to be made from these results are :

(1) That butter with its Fat-soluble A destroyed allowed rickets to develop more rapidly.

(2) That 'casein' (acidic), whether it contained its Fat-soluble quota or not, brought about the development of rickets even though fresh butter was eaten. When the butter had its Fat-soluble A destroyed, the addition of casein brought about severe rickets more rapidly.

(3) There is but little evidence of any real difference in the action of casein whether unextracted or extracted with alcohol. Both were detrimental.

Of these puppies, 321 and 323, both receiving the oxidized butter, never completely recovered from the anaesthetic administered for radiographic purposes, and were killed. The CaO in the shafts of the fresh femurs was abnormally low in both cases, 5.9 (321) and 4.1 per cent. (323).

Having seen that this type of 'casein' hastened the onset of the rachitic condition, it was then removed from the diets of the rachitic dogs to see if recovery would take place and added to the diet of 325 which, up to that stage, had remained normal to see if rickets would develop.

The following alterations were made accordingly in the diets of the three remaining puppies after about 11 weeks of the experiments.

- 322. Bread greatly reduced to see whether, by living for a short time on its own fat stores, recovery would take place in spite of the presence of casein.
- 324. 'Casein' cut out altogether; bread increased to 190 gm. to allow for loss of energy due to removal of casein.
- 325. 'Casein' (15 gm. per diem) added to diet. Bread reduced to 170 gm.

The results of this treatment, as indicated by the radiographs, are as follows:

- 322. (Cutting down bread, 'casein' and butter remaining.) Period of new diet 26 days—weight reduced from 4,910 to 4,390 gm. —puppy remained well. A possible slight attempt at repair resulted.
- 324. (Removal of 'casein'.) Calcification at the epiphyses shows some recovery (Fig. 83).
- 325. (Addition of 'casein'.) Severe rickets rapidly developed (compare Figs. 50, 86, and 87).

On removing 'casein' from the above diet calcification processes were resumed to a slight degree at the epiphyses while the addition of casein brought about severe deficiency of calcification.

It has been shown that edible casein (basic) has but little influence on the development of rickets. When substituted for bread with equivalent energy value, little effect was apparent. The experiments with acidic 'casein' make it clear that this substance depresses calcification processes and hastens rickets even when butter is also eaten. At first these discordant results were most perplexing because it was thought that the casein used in all cases was of the same type. On closer examination it was found that one kind of casein (edible) was alkaline and the second kind acidic. Is the acid attached to the caseinogen the cause of the interference with calcification? It is possible that this acid removes the calcium and prevents its access to the tissues. I do not know how much acid there was in the acidic caseinogen. It was a commercial preparation and such substances often have a great deal of acid attached because of imperfect washing. Even carefully prepared acid caseinogen has a slight acidic reaction,

however long it may be washed after precipitation. It has been shown by Götting (21) that adding oxalic acid to the food of puppies will produce osteoporotic changes in bone. This can be readily understood for it would doubtless combine with any calcium salts of the diet in the alimentary canal so that the outcome would be similar to feeding on foodstuffs deficient in calcium. It has also been stated that lactic acid produced from carbohydrate in the intestine by fermentation is responsible for rickets, but I am unaware of any experimental evidence which supports this view. Although the effect of the acid in acid caseinogen cannot be decided except by further experiment, it does not seem likely to be the real explanation of the above-described facts. Otherwise it might be expected that all the dogs receiving orange and lemon juice would develop rickets, which is certainly not the case.

On estimating the calcium present in the two samples of 'casein', the following results were obtained :

'edible casein'	contained	2.4 per cent.	CaO.
acidic 'casein'	„	0	„ CaO.

The great difference in the various forms of 'casein' on the market is evident from the work of John Mellanby, who has supplied me with the following facts. Caseinogen as present in milk is a protein-calcium phosphate complex of such a nature that 3,600 grm. of protein approximately are associated with one gram molecular weight of $\text{Ca}_3(\text{PO}_4)_2$. On acid precipitation the calcium phosphate is split off and, not only so, but the protein moiety is also changed by the acid. Some of this acid adheres to the protein portion but can be got rid of by careful washing. If for instance hydrochloric acid is used, the precipitate can be washed until no more chlorine remains. The protein after washing is still slightly acidic and decomposes calcium carbonate with the evolution of CO_2 , forming a calcium caseinate of a nature that approximately 7,500 grm. of compound contain 1 grm. molecule of calcium. The acid protein will not combine with calcium phosphate to form the original caseinogen. Acidic caseinogen is in fact an acid owing its acidity no doubt to the arrangement of phosphoric acid in its molecule, and it is most probable that this arrangement is not found in the protein of milk before precipitation.

The protein moiety of caseinogen as found in milk and acid-caseinogen both contain 0.7 per cent. of phosphorus in combination. In milk there is a corresponding amount of calcium. In acid-caseinogen there is no calcium so that the phosphoric acid liberated from it during digestion may be responsible for the deprivation of calcium salts and subsequent defective calcification of bone in the above-described experiments. I have dealt with this question in detail because it is likely that further work will be undertaken by other investigators on the effect of vitamins on calcification processes, and it is obvious how difficult a problem of this nature will be unless the experimenter bears in mind the various actions which may be expected according to the type of 'casein' used in the synthetic diet.

Further, it is evident how important is the caseinogen calcium balance in milk from the point of view of growth of bone.

Since caseinogen, the more abundant protein in milk, contains a large amount of calcium it is not surprising that there is in animals of different species a close relation between protein and calcium content. The total protein-calcium ratio in milk of different animals is, however, not constant for two reasons : (1) the variable amount of lactalbumen ; (2) the variable amount of free di- and tri-calcium phosphate and calcium chloride. If the interpretation of the foregoing experiments is true, the association of caseinogen and calcium is evidently of great importance from another point of view, for any condition which diminishes the amount of calcium in proportion to the protein of milk may also lead to a disturbance of calcium metabolism favourable to the development of rickets. Further experiments will settle this point.

(ii) MILK-FAT.

In dealing with the action of fats, it was remarked that, whereas butter had a stimulating effect on the formation of calcium phosphate in the periosteal bone, occasionally cases of rickets were found where butter was the fat eaten. In these cases defective endochondral ossification was found in bones which subperiosteally were almost normal. Sufficient experiments, perfectly controlled in all detail, have not been made to determine whether butter stands in an exceptional position in this respect as compared with other fats. At first it seemed to me probable that the somewhat discrepant results with butter might be explained by differences in the quality of the butter : when made from summer milk it appeared to be more anti-rachitic than when made from winter milk. Experiments, which will be reported when more data are obtained, were started early in 1919 to test this point. In my experience the problem is more complicated than recent publications dealing with vitamins in milk would indicate.

It is certainly possible to convert a positive calcium balance into a negative balance by an undue increase of butter intake as compared with other foods. In Exp. 155, a puppy eight weeks old was given 15-30 gm. of butter per diem, the rest of the diet being made up of separated milk, bread, and yeast. In spite of the large amount of butter or possibly, because of it, the animal did not flourish and actually lost weight in the experimental period of three months. At the end of this time its bones were very soft and contained only 7.8 per cent. of CaO. There was no indication of rickets revealed by histological examination of the bones. The large amount of butter in the diet had increased the rate of excretion of calcium salts.

It is possible that excess of butter only converts a positive calcium balance into a negative one when it causes a dyspeptic condition to develop. This happened in Exp. 155 described above, where the bones became very soft with a small content of calcium.

That butter in excess has under some conditions the power of increasing the excretion of calcium, and thereby creating a negative balance, is also supported by the experiment of Rothberg (22), Steinitz (23), and Meyer (24). On the other hand Telfer's (25) recent results are contrary to these former workers, for he showed that increasing the butter intake from 21.6 gm. to 43.2 gm.

per diem over periods of five days did not affect the calcium retention in a child. There is probably some simple explanation of the discrepancy between these results because of the general agreement among all the German workers, and it is difficult to imagine serious errors in calcium estimations.

In any case the foregoing type of investigation does not seriously affect either one way or another the work described in this paper, for the period of a few days—three up to seven—during which the metabolic changes were examined by the various researchers is probably too short to lead to results bearing upon the subject of vitamins—at least so far as fats are concerned. For in dealing with the question of fats and the anti-rachitic vitamin, it was evident that several weeks usually passed before a diet deficient in anti-rachitic vitamin showed any obvious effect on calcification when given to a healthy animal.

I have made a few experiments to see whether butter has a greater anti-rachitic action if the calcium intake is increased at the same time. Although this portion of the work is still very incomplete, there is some evidence that butter and calcium salts have a synergistic action in opposing or curing rickets. In dealing with the casein-calcium balance in a preceding section, it was seen that, although butter was eaten, great defect in calcification was produced when 'casein' (acid caseinogen) was added to the diet, and when this protein was removed, attempts at repair and renewed calcification became evident. The rate of this improvement was slow (Exp. 324), and the animal became partially paralysed in its hind limbs. The diet at this period was separated milk 200 c.c., bread 190 gm., butter 15 gm., meat 10 gm., lemon juice 7.5 c.c. On October 12 whey made from 450 c.c. of milk was added to the diet, and by December 1 the rickets was greatly improved (see Fig. 85, taken Nov. 18). The paralytic condition also rapidly disappeared (compare Figs. 84 and 85).

In another puppy of the same series (325) which had bad rickets, having previously eaten 'casein' (acid caseinogen), the 'casein' was removed from the diet and the ash of 45 gm. of separated milk powder added. In six weeks curative processes had been well started. This puppy was also partially paralysed before the salts of milk were added and quickly recovered after their addition (compare Figs. 87 and 88). 322 (same series as 324 and 325), casein (acid caseinogen) was removed from the diet, when advanced, though possibly healing, rickets was present, and 45 gm. of dried separated milk powder substituted for it. In this case the curative processes continued (Figs. 81 and 82). A synopsis of the above curative experiments is as follows :

3 retrievers with bad rickets received.

General Diet :

Separated milk 200 c.c.	Bread 150-190 grm.	
Butter 15 grm.	Lemon juice 7.5 c.c.	
322	324	325
Dried separated milk powder 45 grm.	Whey from 450 c.c. separated milk.	Ash from 45 grm. separated milk powder.

All puppies showed curative changes in six weeks. As compared with the curative changes resulting from the removal of the acid-caseinogen only, it appeared as if the addition of calcium salts either in separated milk powder, whey, or the salts of separated milk powder had hastened the establishment of calcification changes apparent at points of endochondral ossification. These curative changes described above took place although the animals were closely confined, and in two cases the puppies were also paralysed. The paralysis disappeared on change of diet.

These experimental results indicate that butter is a more potent anti-rachitic agent when it has abundance of calcium salts, as found in milk, with which to work. It is but natural that the anti-rachitic vitamine of butter, which certainly has a strong influence on the deposition of calcium phosphate in bone, should also have a sufficiency of calcium salts in the diet before it can work effectively. That it should be necessary to balance closely the intake of butter and calcium salts, so that increase of the former must be accompanied by increase of the latter in order to produce the greatest anti-rachitic effect is not so obvious. The above described results, however, suggest that milk is a foodstuff which is naturally made up of well-balanced constituents, and any artificial changes in the relative proportions of its elements, such as are so commonly carried out for purposes of infant feeding, can only be a safe procedure when our knowledge of this foodstuff is much greater than it is at present.

SUMMARY.

(1) Evidence is offered tending to show that, when casein containing calcium is added to the diet, it has very little effect on rickets. When acid-caseinogen containing little or no calcium is eaten, great defect in calcification of bones is produced, even when the diet contains butter. It is possible that the close relation between the amounts of protein and calcium found in milk of animals of different species is of some importance from the point of view of milk as an article of diet for young animals, and the development of rachitic changes in these animals.

(2) The action of the anti-rachitic vitamine in butter is apparently more effective when there is also abundance of calcium salts in the diet.

(3) These results emphasize the inadvisability of altering the relative proportion of milk constituents in the feeding of infants, until our knowledge of the actions of these components and their interactions with one another are more perfectly understood. To increase either the caseinogen or butter element without at the same time increasing the calcium salts is contraindicated by these experiments. The whole question requires further investigation.

(f) The Relation of Calcium to the Anti-rachitic Vitamine in Foodstuffs.

I wish now to discuss briefly the part played by calcium salts in the diet, and their relation to rickets and conditions simulating rickets.

In the first place the diet must certainly contain a sufficiency of calcium. In the popular mind, and even among medical men and

scientific workers, there is a strong belief that rickets is due to a deficient calcium intake in the food. It may often be an adjuvant factor in the production of rickets, and if other conditions conducive to the disease are present, a deficient calcium intake will certainly exaggerate the signs and hasten the onset of rickets. On the other hand, experimental work has clearly shown that calcium deficiency in the diet alone will not produce rickets. Miwa and Stoeltzner (20) fed puppies on horse flesh and distilled water—a diet very poor in calcium. The changes in endochondral ossification were small, but there was very evident osteoporosis in the shafts of the bone. Götting (21) also produced osteoporotic changes as the result of giving diets poor in calcium. Dibbelt's (9) results are more interesting from the point of view of this research. He fed puppies on horse flesh and fat, and on horse flesh and carbohydrate. In all cases osteoporosis was produced, but when horse flesh and carbohydrate were eaten, there was also an increased amount of 'osteoid tissue'. When fat was eaten with horse flesh, the osteoid tissue was greatly diminished. There seems no doubt that Dibbelt produced a combination of rickets and osteoporosis when there was no fat and anti-rachitic vitamine in the diet, and osteoporosis only when the fat and its vitamine content was eaten. Whether, therefore, osteoporosis or a combination of rickets and osteoporosis develops on diets poor in calcium depends partly on the deficiency in calcium and partly on the other constituents of the diet, excess of carbohydrate favouring the production of osteoid tissue, while the proper type of fat exerts its influence by regulating the correct calcification and structure of any bone that may be laid down.

Just as it is certain that deficiency of calcium alone will not produce rickets, clinical and experimental observations are equally conclusive that abundance of calcium in the diet will not prevent rickets. Certainly the addition of calcium phosphate to a rickets-producing diet will not prevent rickets. In Exp. 122, the puppy—(six weeks old) was given 5 grm. of $\text{Ca}_3(\text{PO}_4)_2$ daily in addition to a diet of 175 c.c. separated milk, white bread *ad lib.*, and 10 c.c. linseed oil. Its weight increased from 1,800 grm. to 3,170 grm. in ten weeks. Bad rickets had developed in this time, and the experiment was stopped after thirteen and a half weeks of the diet. The calcium oxide present in the fresh shaft of femur was 9.3 per cent., and histological examination revealed advanced rickets.

Although the calcium intake cannot be considered a crucial point in the case of rickets, its importance in the dietary can be easily overlooked, for it is a matter of great ease to choose a diet in which the calcium intake is remarkably low. A diet of this nature would include all forms of meat, white bread, margarines and most other fats, sugar, potatoes, polished rice and many manufactured cereals. These substances form the basis of the normal diets of many people, especially the poor in this country, and it would be surprising if a deficient calcium intake were not a very common defect. The amount of calcium reckoned as calcium oxide is found in the following foodstuffs: ¹

¹ Figures calculated from those of Albu and Neuberg (*Mineralstoffwechsel*, Berlin, 1906).

	%		%
Meat	0.015 CaO	Cow's milk	0.16 CaO
White bread	less than 0.014	Egg yolk	0.4
Sugar	none	Cabbage	0.45
Margarine	none	Cauliflower	0.14
Potato	0.025	Butter	0.37
Polished rice	0.016	Oatmeal	0.12

In this table I have contrasted the foodstuffs containing abundant calcium with those with very little calcium.

Even assuming that foodstuffs are passive as regards calcium metabolism, so that all the calcium salts absorbed from the intestines are stored up in the body, it is obviously easy, especially in those places where the water is almost free from calcium salts, to eat a diet which can only result in the defective calcification of bone. The osteoporotic condition that develops in dogs from a deficient lime intake produces an external appearance identical with rickets. It seems to me probable that deficient calcium in the food may play a more important part in the aetiology of rickets—more especially in late rickets so common nowadays—than is sometimes assumed. For it is obvious that, however favourable the diet and metabolic conditions may be, they must be inadequate from this point of view if there is little or no calcium absorbed. On the other hand, if other dietetic conditions favour the production of rickets, a deficiency of calcium in the food will emphasize the pathological changes.

What then is an adequate calcium intake? There is no absolute amount that can be described as adequate, because the amount necessary for the production of perfect calcification has been seen to depend on the other elements of the diet and environment, some of these factors helping the cells of the body to make use of calcium while others prevent this action. It is all a question of balance.

It is a most fortunate occurrence that nature has so ordered matters that if an attempt be made to eat foodstuffs containing an abundance of calcium, those substances generally also contain a large amount of the anti-rachitic vitamine, or, in any case, the Fat-soluble A vitamine. Milk and egg yolk contain a large quantity both of calcium salts and anti-rachitic factor. Cabbage and other green vegetables are very rich in calcium and also Fat-Soluble A—in fact green leaves are undoubtedly the original source of this vitamine. It has so far not been possible in this investigation to carry out successfully any experiments on dogs to decide whether green leaves have a potent anti-rachitic effect, but it can be prophesied with some safety that this will prove to be the case. The opposite condition also holds with many of the commoner natural foodstuffs, for those that contain no anti-rachitic vitamine so far as our present knowledge goes also have a low calcium content. Not only, therefore, do the experimental results obtained in this research point to a close interworking between the anti-rachitic vitamine and calcium salts, but also their distribution in nature would give support to the same view.

SUMMARY.

An analysis of ordinary articles of diet shows the ease with which diets deficient in calcium can be chosen. Although a deficient intake of calcium alone is not the cause of rickets it is probable that this is

a common cause of exaggerated signs and symptoms in the disease, and more particularly in late rickets.

The choice of a diet rich in calcium, e. g. milk, egg yolk, and green vegetables, fortunately leads to the ingestion of an abundance of Fat-soluble A, and substances deficient in one are usually deficient in the other. This distribution in nature indicates a close interaction of these two substances, and supports the experimental results obtained.

V. THE PART PLAYED BY EXERCISE AND CONFINEMENT IN THE AETIOLOGY OF RICKETS, AND THEIR RELATIVE IMPORTANCE AS COMPARED WITH DIET.

Mainly as the result of observations made on wild animals when confined in zoological gardens, Hansemann (31) suggested the 'domestication' hypothesis as the cause of rickets. 'Domestication' factors included various conditions such as confinement, lack of exercise, and absence of fresh air, and was too comprehensive to convey much meaning. The hypothesis was narrowed down by Findlay (13), following some experimental work on the development of rickets in puppies, which, while remaining on the same diet of porridge and milk, were either confined to rooms in the laboratory or allowed to run about and take exercise in the open. The experimental results were in favour of confinement being the real cause of rickets. Findlay's experiments I have criticized elsewhere, and it only remains to state here that, in my opinion, the diets were neither good enough nor sufficiently controlled to allow of the important conclusion that lack of exercise alone is the cause of rickets in children. The experiments on the effect of confinement were repeated by Paton, Findlay, and Watson (26), and the results obtained were in agreement with those of the earlier work. In these latter experiments the diets were more closely controlled, and attention was paid to my previously published conclusion that a vitamine of the nature and distribution of Fat-soluble A played a part in the aetiology of rickets. These workers got no evidence that such was the case, and the main factor influencing the development of rickets in their experiment was exercise.

In addition to animal experiments, a large enquiry into the cause of rickets in children was carried out in Glasgow by Ferguson (32), Findlay, and Paton, a great deal of data as to housing and diet being collected in order to discover why some families were rachitic and others normal. The conclusions reached in this investigation were in agreement with their previous animal experimental results, for it was found that there was close correlation between the number and size of rooms used for habitation and incidence of rickets, and no evidence of a dietetic factor playing a part in rickets was obtained. This research I have also criticized elsewhere (1 and 2).

As the result of the two types of work carried out in Glasgow, Findlay has come to the conclusion that lack of exercise is the cause of rickets. Paton now disagrees with Findlay that exercise plays this predominant part, and thinks there is some other factor possibly

of an infective nature to be considered. This latter view he does not deduce from his own experiments only, but lays stress on the observations of Bull, who described an 'epidemic' of rickets among foxhound puppies, the only certain cure of which was to remove the puppies to some part of the property where they had not previously been. It ought to be added that Bull (29) thinks diet also of importance, and states that he believes that 'had more opportunity occurred of improving the diet, the disease would have been completely controlled'. The infective hypothesis of rickets has been advanced by others, including Morpurgo (35), J. Koch (27) and Marfan (28), and I shall discuss it later. At this point I may say that I do not consider that infection has anything to do with rickets in a normal way, but it may hasten the development of symptoms and exaggerate the disease if the other rickets-producing conditions are present.

As regards the effect of confinement and exercise, I shall now show that, although exercise has an undoubted anti-rachitic action in the case of puppies, its importance is quite subsidiary to diet, and that even the possibility of taking exercise is dependent on diet.

The effect of confinement on the development of rickets was one of the first things tried in this research, at a time when I had no ideas as to the causative factors. It will be noted that the earlier experiments are crude in that no quantitative data are known as to the different constituents of the diet eaten. On the other hand, the conditions of confinement were strenuous, and the complete absence of rickets in these puppies led me to the conclusion that confinement did not hold the important position in the aetiology of rickets that Findlay had ascribed to it.

In later experiments full details of diet are given, and with variation of diet it is possible to see in better perspective the relation between confinement and diet in the development of rickets. The subject is by no means fully worked out, but sufficient data are at hand to afford the opportunity of appraising the value of these two factors.

Experimental work has been carried out on various lines, including the following :

1. Puppies have been confined but allowed a good diet. They have not developed rickets.
2. Puppies have been allowed complete freedom during the daytime, but the diet has been defective. In all cases these puppies have developed rickets. Only one has remained almost normal. This puppy was of a small breed, ate but little food and grew very slowly during the experimental period.
3. Animals have been given rickets and later have been confined, while at the same time the diet has been altered so as to contain a supply of anti-rachitic vitamine. It will be seen that in spite of confinement curative processes started.

Each of these points will be treated in turn.

(a) **The Absence of Rickets during Confinement on a good Diet.**

In the earliest experiments which were made on this point four puppies were placed on the following diets :

- (a) Bread, milk and meat.
- (b) " " " and bone.
- (c) " " " and bone that had been autoclaved (120° C. for a quarter of an hour).
- (d) Bread, milk and meat and calcium phosphate (10 grm. per diem).

The animals were confined absolutely in kennels 3 ft. 6 in. by 2 ft. 6 in. They were 10 to 12 weeks old at the commencement of the experiment and after 13 weeks confinement were killed (Exp. 25, 26, 27, 28).

No. of experiment.	Initial weight. gram.	Final weight. gram.	Calcium oxide in dry bone. %
25 . . .	3,280	4,438	42.92
26 . . .	4,280	6,680	38.13
27 . . .	3,300	6,460	37.46
28 . . .	2,810	4,030	30

Histologically no sign of rickets could be seen. Chemical examination revealed the calcium content of the shafts of the bones to be high.

The criticism against these experiments is that the animals were 10 to 12 weeks old at the beginning of the experiment. Had the confinement delayed calcification, however, there would have been evidence of this in the smaller calcium content of the shaft of the bone (*vide* Exp. 264, 265, 266, and 267, p. 26).

The following Exp. 29, 31, and 32 were carried out on similar lines. Puppies 31 and 32 were of the same family.

Exp. 29. *Diet.* Bread.
Milk.
Meat.

Length of experiment. 18 weeks.

Initial weight. 2,255 grm.

Final weight. 7,150 grm.

Calcium oxide in dry bone. 35.84 per cent.

Histology of bones. Normal.

Exp. 31. *Age.* 8 weeks old at beginning of experiment.

Diet. Bread. Changed after 13 weeks to :
Milk. Bread.
Meat. Milk.
Calcium phosphate. Melox.

Length of experiment. 22 weeks.

Initial weight. 2,150 grm.

Final weight. 6,810 grm.

Calcium oxide in dry bone. 36.64 per cent.

Histology. Normal.

Exp. 32. *Age.* 8 weeks old at beginning of experiment.

Diet. Bread.
Milk.
Meat.
Autoclaved bone.

Length of experiment. 22 weeks.
Initial weight. 2,708 gm.
Final weight. 6,580 gm.
Calcium oxide in dry bone. 32.36 per cent.
Histology of bones. Normal.

In the following experiments the diets were more closely controlled.

Exp. 156. *Age.* 5 weeks old at beginning of experiment.
Diet. Whole milk 300 c.c. Raised to 400 c.c. after 10 weeks confinement.
 Meat 50 gm.
 Yeast 10 gm.
 Wheaten bread 70 per cent.
 Linseed oil 5 c.c.
Length of experiment. 18 weeks.
Initial weight. 1,695 gm.
Final weight. 6,660 gm.
Calcium oxide in dry bone. 25.40 per cent.
Calcium oxide in fresh bone. 17 per cent.

No rickets. Histological examination shows bone normal.

Exp. 190. *Age.* 6 weeks.
Diet. Bread *ad lib.*
 Yeast 5 gm.
 Salt 1 gm.
 Orange juice 3 c.c.
 Separated milk 175-250 c.c.
 Cod-liver oil 10 c.c.
Length of confinement. 7 weeks.
Initial weight. 1,325 gm.
Weight after 7 weeks. 2,315 gm. Legs bent early in experiment owing to the loosening of the ligaments.
 Animal ran very well and was lively.

Radiograph at this period shows normal bones (Fig. 10).

Radiographs of 186, 187, and 189, puppies of the same litter as 190 kept under the same conditions, of which 186 had linseed oil, 187 had cotton-seed oil, 189 had peanut oil, show that during this period of confinement they had developed rickets (Figs. 7, 8, 9).

CaO in fresh bone 14.5 per cent.

Note.—In Exp. 190 the diet contains no *Meat*.

Exp. 198. *Age.* 8 weeks at beginning of experiment.
Diet. Bread *ad lib.*
 Whole milk 250 c.c.
 Meat 20 gm.
 Cod-liver oil 5 c.c.
 Orange juice 5 c.c.
 Salt 1 gm.

Length of Confinement. 13 weeks. At the end of this time the legs were quite straight and the X-ray photograph was normal (Fig. 89). Animal runs well.
 A photograph of this puppy is shown in Fig. 90.
Initial weight. 1,405 gm.
Weight after 13 weeks. 5,925 gm.

This puppy did not develop the loose ligaments seen in the previous experiment (190) and, throughout the period of confinement, remained a fine healthy animal with a beautifully glossy coat.

Exp. 350. 8 weeks old at beginning of experiment.

Diet. Whole milk 200 c.c.

Melox 50-150 gm.

Meat 20 gm.

Length of confinement. 14 weeks.

Initial weight. 1,280 gm.

Weight after 14 weeks. 4,000 grms.

Radiograph (Fig. 113) is normal.

This animal is still alive and is in perfect health.

Here we have a number of experiments carried out with confined animals. In all cases the diets contained abundant anti-rachitic factor, and except for the slight looseness of the ligaments seen in 190, which was otherwise a normal puppy, there has been no suggestion of the development of rickets. On the whole it will be seen that the animals grew well and kept in perfect condition.

(b) Development of Rickets when Exercise is allowed on a Defective Diet.

Exp. 192. } Terriers.
193. }

Diet. Bread *ad lib.*

Yeast 5 gm.

Salt 1 gm.

Orange juice 3-5 c.c.

Separated milk 250 c.c.

Linseed oil 10 c.c.

Of these two puppies 192 was allowed complete freedom during the day, 193 was confined.

It will be noticed that the diet in these two experiments was of a ricket-producing nature.

Initial weights. 192. 1,980 gm. *Final weights.* 192. 3,275 gm.

193. 1,450 gm. 193. 2,660 gm.

Length of experiment. 19 weeks in both cases.

X-ray photographs taken after 17 weeks of diet show that both puppies had developed rickets, 193 (Fig. 95) (no exercise) being worse than 192 (Fig. 96).

The deduction from these experiments and others of the same type is that confinement has made the condition worse, but that, even with the full possibility of exercise, well marked characteristic signs of rickets developed. Both these dogs were ill towards the latter end of the experiment and 193 died. A similar outcome would probably have resulted in 192 had not the diet been altered by substituting cod-liver oil for linseed oil and the addition of 50 gm. of meat for a few days. The subsequent history of this dog 192 is referred to elsewhere (p. 65).

Exp. 199. This puppy was of the same family as 198 (*vide supra*); both being terriers.

Animal allowed complete freedom.

Age. 8 weeks at the beginning of experiment.

Diet. Bread *ad lib.*

Yeast 5 gm.

Salt 1 gm.

Orange juice 5 c.c.

Linseed oil 10 c.c.

Separated milk 250 c.c.

Marmite 1 gm.

Length of experiment. 19 weeks.

Initial weight. 1,485 gm.

Final weight. 4,440 gm.

A series of radiographs of the wrist of this puppy are shown (Figs. 92, 93, and 94). It will be seen that rickets developed after 10 weeks. During the continuation of the experiment under the same conditions, the radiographs show improvement in the rachitic condition. This is an instance of self cure occurring while the animal remained on the same diet. Possibly the larger amount of separated milk given to this animal played some part in recovery.

Calcium oxide in fresh femur (after healing process at epiphyses established), 6.91 per cent.

In spite of the healing process, evident in the radiographs, the calcium in the bones has remained very low. The amount of rickets was certainly not very profound, but on the other hand, the dog was of a small type which grew slowly as compared with 198. The dog's bones were bent to some extent and its external appearance was distinctly rachitic. It must be doubted, however, whether the healing process would have started and continued in this fashion, had not the dog been allowed complete freedom. The very low calcium content of the bone, which apparently did not increase to any extent, in spite of the healing changes evident at the epiphyses, emphasizes the danger of laying too much stress on radiographic examination as evidence of cure. That the condition, as the result of the continuation of the experiment, improved, is undoubted, but the small amount of calcium oxide present in the shaft of the bone, indicates that the improved conditions of calcification that were resumed after the disease had developed were slight in quality and did not have sufficient time to affect the periosteal bone.

Exp. 165. In this case also the dog was allowed freedom and took much exercise during the experimental period.

Small terrier breed of dog.

Age. 8 weeks old at beginning of experiment.

Diet. Bread 70 per cent. *ad lib.*

Separated milk 175-250 c.c.

Linseed oil 10 c.c.

Yeast 10 gm.

Orange juice 5 c.c.

Meat occasionally.

Length of experiment. 17 weeks.

Initial weight. 1,330 gm.

Final weight. 2,900 gm.

In the eleventh week of the experiment the animal's weight was only 2,200 gm.

This animal had only slight rickets when examined histologically.

Calcium content in dry bone. 29 per cent.

Calcium content in fresh femur. 18.85 per cent.

Exp. 164. As compared with a brother of the same litter, 165, which also did not grow much, the exercised dog was certainly more normal. 164 was on a similar diet to 165 but was kept confined throughout the experimental period.

Initial weight. 1,340 gm.

Final weight. 2,300 gm.

Maximum weight. 2,475 gm.

Histological examination shows it to have rickets.

Calcium content in dry bone. 17.9 per cent.

Calcium content of fresh femur. 10.7 per cent.

In comparing these two dogs 164 and 165 it is clear that the exercise has retarded the development of rickets. The smallness of the dog (165) and the slight rate of growth would, from previous experience, indicate that no great accessory influence would be necessary in this case to turn the balance from the rachitic to the non-rachitic condition. The amount of exercise received by the dog was apparently of sufficiently great influence to keep it almost normal from the rickets point of view. It was most difficult, especially in the first ten weeks of the experiment, to get these dogs, particularly 165, to eat their food. 165 drank the separated milk with avidity, but preferred, as a rule, to leave the rest of its food.

Exp. 251. } These were retriever puppies.
252. }

Age. 6 weeks old at start of experiment.

251 and 252 were put on the following diet :

Bread 100-200 gm.

Separated milk 175-250 c.c.

Yeast 10 gm.

Orange juice 5 c.c.

Linseed oil 10 c.c.

Meat 10 gm.

251 had no exercise, 252 was out continuously in the open air.

Initial weights. 251. 2,180 gm.

252. 2,210 gm.

Weight after 9 weeks. 251. 5,020 gm.

252. 4,875 gm.

Radiographs of these puppies taken after nine weeks of the diet are shown, and it will be seen that both have developed advanced rickets, but that of the two, 251 (Fig. 102) (no exercise) is worse than 252 (Fig. 104).

In this case we see, again, that exercise has had an improving

effect on the condition so far as radiographic examination is concerned, but that unlike 165, where we were dealing with a small type of puppy eating very little bread, the exercise obtained in this case has not prevented the development of bad rickets.

In the above described experiments made to test the effect of exercise and confinement, meat usually formed an element of the diet in the confined animals and was absent in that of the exercised animals. In Findlay's experiments (13) and in the later work of Paton, Findlay, and Watson (26), no meat was eaten. It seemed possible that some part of the discrepancy between our results might be due to the presence and absence of meat in the respective experiments. In another section of this paper I have dealt with the action of meat in rickets and shown it to be definitely but slightly anti-rachitic, this action being more noticeable as regards endochondral calcification than calcification of periosteal bone when the diet is deficient in the anti-rachitic vitamine.

It is of interest to note in this connexion that Lehnerdt (30), working on the effect of confinement, also came to the conclusion that it was not the cause of rickets. The account of his work is brief and contains but little detail. Of six puppies, five weeks old, three were allowed a two-hours' run daily in a garden and, at other times, were closely confined together with the remaining three which got no opportunity for exercise. After some months of this treatment no evident rickets was produced in any of the dogs. The bones of the confined animals were hard and macroscopically showed no rachitic changes. Microscopically they were not examined. The food eaten by the puppies consisted of milk, meat, and bread, and each puppy was given 1 grm. of calcium phosphate daily. It will be noticed that this diet differs from that used by Findlay in that it contained meat and is, in fact, the same diet as that used in my earlier experiments, when confinement did not produce rickets.

In the section dealing with meat I have described experiments made with the object of seeing what is the effect of adding meat to a rickets-producing diet when animals are confined, and when allowed full freedom during the daytime (with muzzles on) (see Exp. 351, 352, 353, and 354). Experiments on diet and exercise are useless unless the puppies are muzzled. Instinct leads them to know what their diets lack and they will make good this deficiency if given the opportunity. Of these animals 351 and 352 were allowed freedom, 353 and 354 were confined. The diets were exactly the same, quantitatively and qualitatively, in all the dogs except that 351 (exercise) and 353 (confined) were given an extra ration of lean meat (20-30 grm.) per diem. After eight weeks both the puppies getting no meat, confined and with exercise (352 and 354), developed rickets, easily recognizable in the radiographs, Figs. 63 and 65. Neither of the meat-eating puppies (351 and 353) showed definite rickets by radiograph at this stage of the experiment (Figs. 62 and 64). The anti-rachitic effect of the meat is evident both when the animals are confined and allowed exercise. It may be added that, throughout the whole experiment, all these puppies lived out of doors. The effect of adding meat to the diets requires further comment. The difference in the behaviour of the two free puppies

(351 and 352) was most noticeable, 351 (meat) ran about all over the laboratory grounds and showed great interest in the activity of the workers, following them from place to place. 352 (no meat), although given the same facilities as 351, developed a striking lethargy in contrast to 351, and unless stirred up to make an effort preferred to meander slowly round. No better example could be seen of the dominant effect of diet in controlling the activity of an animal.

As regards the confined puppies (353 and 354) neither of these were capable of running, and at first sight 353 (meat) looked as rachitic as 354 (no meat), the legs of both being bent. A closer examination, however, soon revealed that the bending of the legs of 353 was largely ligamentous and that, in contrast with 354, there were no obvious epiphyseal swellings of the leg bones.

The weight curves of these four animals seen in Fig. 66 show that the diets were closely controlled. We see from these results (351, 352, 353, and 354) that

- (1) Complete freedom during the daytime has not prevented severe rickets from developing (352).
- (2) Meat had a definite anti-rachitic effect both when the animals were confined and when taking exercise.

It is evident that the presence of meat in the diet may explain some of the discrepancies between the various results obtained by different workers on the effect of confinement. I have shown, however, in dealing with this anti-rachitic action of meat that it is small, and bad rickets can be produced in quickly growing puppies even when 50 grm. of lean meat are eaten daily if the diet is otherwise bad.

There seems to be something in common between the anti-rachitic effect of meat and exercise. When the animals are on poor diets (with abundant bread and deficient in foods containing the anti-rachitic vitamine) neither exercise nor meat singly seem to have any potent effect. Acting together, even when the diet is poor, their effect is much more strongly anti-rachitic. With moderate diets, both meat and exercise are capable of putting the animal into a safe position.

In some experiments the animals have not been allowed either meat or exercise and no rickets has developed. The diets in these cases have contained plenty of the anti-rachitic vitamine and calcium salts; the bread has usually been kept low. During the past year it has been my custom to keep all the puppies confined to their kennels, except when the effect of exercise has been tried, and, if confinement holds the important position in producing rickets that some think, none of my experimental puppies during this period ought to have escaped the disease. This, of course, is not the case.

(c) **The Curative Effect of Substances containing the Anti-rachitic Vitamine when the Animals are confined.**

It has been pointed out above that as the animals grow older curative changes are sometimes set up in rachitic bones without any change in the diet. It must, therefore, be a matter of difficulty to decide what part age in itself, as compared with alteration in the

diet, plays in any curative changes that may be obvious. In the cases already mentioned, where radiographic examination makes it clear that calcification at the epiphyses has resumed and improvement followed without change of diet, the extent of the rachitic changes, although undoubted, were not excessive.

In order to reduce the curative effect that attaches to increasing age to its lowest limit I have taken two precautions :

1. The animals chosen had rickets, generally of a very severe nature.

2. During the period when the effects of an element of a diet were being tested, the animals were kept confined, so as to eliminate any beneficial effect which we have already seen exercise to have. In several of the animals the rickets was so bad when the curative diets were given that they were completely immobilized by paretic hind legs.

The effect of Milk. The beneficial effect of milk on the disease of rickets can be well seen in Exp. 220. The radiograph (Fig. 15) of this animal shows it to be a case of extreme rickets. One month after the previous radiograph the second photo was taken (Fig. 16). In the short period of one month the curative process has been obviously started and calcification changes have been resumed at the epiphyses. This change is synchronous with the exchange of whole milk for the separated milk previously in the diet.

Exp. 192. This dog also developed rickets while on a rickets-producing diet containing linseed oil. The experiment has been described in an earlier section on the effect of exercise (see p. 60), for this animal developed rickets while being allowed exercise. The diet was changed, cod-liver oil being substituted for linseed oil. 50 gm. of meat were also added for a few days and then reduced to 20 gm.

The effect of making this alteration in the diet was almost instantaneous. The animal became much more lively and the curative process in the bones commenced. These curative changes can be seen by comparing the radiographs of Figs. 96, 97, and 98. This animal was not confined during the curative period, but at the beginning of the cure it was almost incapable of movement.

The effect of Egg Yolk as compared with Egg White.

Exp. 250, 251, 252. All developed severe rickets in the course of an experiment (Figs. 100, 102, and 104).

Two months after the beginning of the experiment, the yolks of two eggs were added to the diet of 250, the white of two eggs to the diet of 251, while that of 252 remained unaltered, viz. the control rickets-producing diet without addition.

A series of radiographs of these three puppies (Figs. 100, 101, 102, 103, 104, 105) shows that the egg yolks have resulted in the recommencement of calcification processes in the case of 250. 251, with the addition of egg-white, remained in a stationary rachitic condition. 252 on the rickets-producing diet steadily grew worse.

We must assume from this experiment that egg-yolk contains an abundance of anti-rachitic vitamine, but that egg-white is free from this substance.

The effect of Cod-liver Oil.

Exp. 205. A greyhound puppy was put on 15 c.c. of cod-liver oil per diem instead of linseed oil, after it had developed severe rickets (Fig. 106). It was also kept indoors in a cage and got no exercise. The curative changes resulting from this alteration in diet were immediate, as can be seen from the radiograph of Fig. 107.

The effect of Butter.

Exp. 213. This puppy developed rickets on a diet in which crude rape oil was the essential fat (Fig. 108).

After about fourteen weeks of the rickets-producing diet, 20 gm. of butter per diem were substituted for the rape oil. Here again it will be seen from the radiographs of Figs. 108 and 109 that curative changes followed on the alteration of the diet by the substitution of butter for the crude rape oil.

Reviewing all these changes described above it would appear that certain articles of diet are able to bring about a profound improvement in the rachitic condition, even when exercise ceases to be an element of importance and where it is highly probable the animals would have died, possibly of extraneous disease, had not the food-stuffs eaten been changed.

The substances having a curative effect include whole milk; meat, yolk of egg, butter, and cod-liver oil. The curative changes described above are doubtless due to the alteration in diet and not to any extraneous factor. It is remarkable how rapidly curative changes commence, so that even in one month in most cases there is considerable renewal of calcification at the epiphyses. How long it would take to bring about hardening of the periosteal bone cannot be stated, but it is probable that this change is a slower one. It is certainly true that renewed calcification of the epiphyses of rickety bone is a rapid and delicate process which can be effected in the curative direction very readily by diet.

Experiments described in this section show that :

- (1) Puppies on good diets containing abundant milk, cod-liver oil, and meat have not developed rickets in spite of strenuous confinement.
- (2) Puppies when allowed freedom during the daytime have developed rickets when the diet has been strongly rickets-producing, i.e. consisting of bread, orange juice, yeast, linseed oil, and a limited amount of separated milk.
- (3) Puppies with very bad rickets have had calcification processes of a curative nature set up at the ends of their long bones even when confined, by the addition to their diets of whole milk, butter, cod-liver oil, and egg-yolk.
- (4) Even the amount of exercise a puppy takes depends on the adequacy of the diet from the qualitative point of view.

In view of these results, therefore, I suggest that the rickets-producing influence of confinement is of secondary importance to the effect of defective diets, even in the case of puppies. When the disease is considered in relation to children, any general review of the facts only emphasizes the relative importance of the dietetic as

compared with the confinement factor. In this consideration the following points may be suggested :

- (1) During infancy and the period of life in which rickets most frequently develops, a child spends a large part of its time in sleep. Up to the age of one it can only make the most limited movements, and the exercise it gets consists of its small movements, including restlessness and crying. During this period, as indeed throughout life, its general activity depends on the adequacy of its feeding and not on the amount of space wherein it can move about. If due to lack of exercise no child ought to escape rickets, more especially those children of the well-to-do who spend most of their days in cots, nurse's arms, and perambulators. It is indeed curious that symptoms of rickets usually become more prominent after the age of one, at which time the average child is capable of moving about and therefore gets more exercise.
- (2) It will be agreed that, although rickets is more rife to-day than at any period in the world's history, conditions of housing are certainly not worse now than they have been. If the exercise a child gets depends on the size of house it occupies, then rickets ought to be less now than in our ancestors' time when the conditions of housing and general hygiene were infinitely worse.
- (3) As I have pointed out elsewhere (2) dreadful housing conditions and most unhygienic surroundings are found in the Island of Lewis in the Hebrides. Not only so, but the children rarely leave the 'Black' houses in these Islands until they can walk at the age of about one year. In spite of the unhygienic conditions and lack of exercise and fresh air at an early age, the children of these islands are free from rickets, having very good teeth and an abnormally low infant mortality. Between the ages of one and five the mortality is high, largely due to tuberculosis. The same conditions surround Esquimaux children, and here again rickets is absent and splendid teeth are developed. In both of these instances the diet of the people contains much anti-rachitic vitamine, because of the amount of fish and blubber eaten. Of great importance also is the fact that the children are breast-fed.
- (4) No sudden cessation of exercise *per se* can explain the extraordinary amount and severity of rickets that has recently developed in Vienna and other towns in Central Europe in consequence of the war. The problem is obviously a dietetic one and depends largely no doubt on the absence of fats and milk in these countries. It is true that the children are described as lethargic, but the lethargy and lack of growth must be secondary to the abnormally bad dietetic conditions.

I have dealt with the anti-rachitic effect of exercise and its relation to rickets in some detail because, although it is obviously an important factor, it would be calamitous if exercise alone were

regarded as giving immunity to rickets or if it were supposed that a solution of the housing problem were going to eradicate rickets from our midst and confer on the people sound teeth. The housing of all people under good conditions with greatly improved hygienic surroundings is a desirable object, but the real solution to these problems of health is without doubt prominently dietetic. A good diet will itself increase the activity of the young, whereas a rickets-producing diet made up of much bread and little milk will take away any tendency to great activity that an animal may exhibit.

The fact that exercise should have some anti-rachitic action is in physiological accord with the dietetic effect described above, and does not, to my mind, involve the break in continuity that some people imagine. As regards the diet, two outstanding factors are prominent, viz. :

- (1) That some fats have a potent anti-rachitic action.
- (2) That other foodstuffs, more particularly cereals like bread, have a rickets-producing effect.

It is certain that lack of exercise induces the laying on of fat, which is formed partly from the fat eaten, but, to a much greater extent, from the carbohydrates of the food. Whatever its origin the laying on of fat involves the immobility of the anti-rachitic vitamine which is stored with the fat in the connective tissue. Exercise burns up the fat and prevents the excessive conversion of carbohydrate to fat, and therefore gives the body the opportunity of making full use of the vitamine content of the diet.

Another influence of exercise is on the energy-bearing elements of the diet like bread. Other conditions being equal, the greater the amount of bread and other cereals, the more rapidly do the animals grow and put on weight. The greater therefore is the demand on calcium salts and the substances in the body controlling calcification processes. There is, in consequence, a greater laying down of non-calcified bone (osteoid tissue), and rickets results. Exercise hastens the combustion of bread and other energy-bearing substances with a like action, and these substances are afforded less opportunity of participating in the formation of the body tissues.

These two effects of exercise on the metabolism, viz. the prevention of demobilization of the anti-rachitic vitamine in the *dépôt* fats and the prevention of energy-bearing substances like bread from participating to an inordinate extent in the building up of body tissues, account for, no doubt, some part of its anti-rachitic action. In view, however, of the experimental results obtained with dried thyroid, described below, which also stimulates the metabolism, I am inclined to think that exercise, in inhibiting rickets to some extent, acts also in a way which is not at present understood.

SUMMARY.

Exercise has an anti-rachitic effect in puppies, but this action is not only subsidiary to diet, but even the possibility of exercise is directly dependent on the quality of the diet. It has been shown above

- (1) That puppies during absolute confinement will not develop rickets if the diet is good.
- (2) That puppies allowed unlimited freedom will develop rickets on the rickets-producing diets used in these experiments.
- (3) That rachitic puppies in confinement can be cured by the addition to the diet of whole milk, cod-liver oil, and egg yolk (substances rich in the anti-rachitic vitamine), and more especially if meat is also present in the food.

Reasons are given which indicate that the effect of exercise on rickets in children as compared with puppies is even of less account.

VI. THE EFFECTS OF ADDING THYROIDEUM SICCUM TO RICKET-PRODUCING DIETS

In an earlier publication I suggested that the anti-rachitic effect of exercise might be due to the increase of metabolism that it brings about. This would prevent the conversion of carbohydrate to fat and the deposition of the latter in subcutaneous dépôts. Demobilization of anti-rachitic vitamine would thus be inhibited. Besides ensuring better activity of this vitamine, it would diminish the influence of bread and other energy-bearing material deficient in anti-rachitic vitamine, in so far as the structure of new tissue was concerned.

It was further suggested that the anti-rachitic effect of meat might be to some extent explained by the increase in metabolism that it causes.

If this were the whole explanation of the anti-rachitic effects produced by exercise and meat, it follows that any method of increasing metabolism would have the same action. One of the best methods of increasing the metabolism is by giving thyroid gland to eat. Consequently a few experiments were made to test the effect of thyroid feeding on the development of rickets.

The following experiments show that adding thyroid to a rickets-producing diet has little or no effect on the development of rickets under the conditions described.

Effect of thyroideum siccum.

General Diet.

Exp. 342 and 343 ate

Separated milk 175 c.c.

Bread 50-150 grm.

Yeast 5 grm.

Salt 1 grm.

Lemon juice 5 c.c.

Olive oil 10 c.c.

Meat 15-20 grm.

Terriers. Age at beginning, 7 weeks.

Outside kennels throughout. No exercise.

In addition 342 received dried thyroid varying between 5 and 30 grains per diem—on most days 10 grains or more. It will be noticed how large were the doses of thyroid given.

The energy of the diets of the two puppies was kept constant for

seven weeks of the experiment, after which period the thyroid dog did not finish up its ration. By this time, however, the results obtained by radiograph made it clear that the thyroid had not prevented rickets.

The radiographs of 342 and 343 after twelve weeks of feeding are seen in Figs. 110 and 111. The weight curves are shown in Fig. 112. It is obvious, since the energy of the diets was the same, and the animals were living under strictly comparable conditions, that the thyroid had increased the oxidation processes of 342, for its increase in weight is much smaller than 343. In spite of this increase in metabolism there was no well-defined anti-rachitic effect present.

Whether thyroid would exhibit an anti-rachitic action if the diet contained a fat with anti-rachitic vitamine such as butter has not been tested. Possibly it would do so, but with olive oil as the sole fat of the diet the effect is apparently not produced. Also when linseed oil was the fat of the diet, thyroid by mouth did not prevent rickets.

SUMMARY.

The increase in metabolism produced by exercise and meat cannot alone explain their anti-rachitic effect, for the addition of thyroid gland to the diet, while increasing the metabolism, did not produce any large anti-rachitic action.

VII. THE HYPOTHESIS THAT RICKETS IS DUE TO AN INFECTION

Among the many causes that have been put forward as explaining the development of rickets, infection finds a place, and even at the present time this hypothesis is viewed with favour by some.

In recent times the work of Morpurgo (35), J. Koch (27), Marfan (28), and Bull (29) has been advanced in favour of the view that rickets has an infective origin.

The results of this extensive research lend no support to this view. Animals have been kept under all possible conditions, both of segregation and close contact, and I have never made any observations that would lead me to believe that the results were being influenced by an extraneous circumstance such as infection. It appears to me to be probable, however, that illness of any kind acts in the direction of producing rickets, so that a border-line case may develop rickets, and slight rickets be made worse if illness, more particularly an illness giving rise to pyrexia, intervenes. I can offer no evidence in support of this statement, which is only an impression produced by observing animals when suffering from distemper and other disorders.

It is possible that the explanation of the action of the illness in these cases is that the animals were generally more closely confined in warm rooms, and even if this were not the case, they spent most of their time lying at the back of their kennels. Apart from this factor, however, there can be but little doubt that calcium meta-

bolism is gravely deranged during pyrexia. Excellent evidence of this is afforded by the appearance of human teeth defects easily observable after eruption in the enamel—bands and pitting appearing in that enamel which was being laid down during the illness. It is probable that the cessation of calcification processes at these times is an indication that calcium is being used elsewhere in assisting the tissues of body to defend themselves against toxic agencies, for there seems no doubt that the addition to a diet of plenty of whole milk containing, among other things, abundant calcium salts and anti-rachitic vitamine, not only has desirable curative effects, but also plays a large part in actually preventing disease. While, however, there is good reason for thinking that an infectious illness interferes with the calcification of bone, and might, therefore, either increase the conditions suitable for the development of rickets or even make the disease worse, it is quite another matter to describe rickets as due to an infection. Rickets is a notoriously 'healthy' disease, very insidious in nature, and is often found in animals and children which, at first sight, are not only well but fat and flourishing and taking their food with an appetite. It is only later, when the disease is developed, that a distaste for food is seen and the animals begin to lose weight. It is, also, after the appearance of rickets, that catarrhal conditions and great susceptibility to intercurrent infection develops, although, of course, these may be found quite independently of rickets.

It is impossible for me to believe that the drastic treatment of J. Koch (27) to produce rickets in puppies, in which work he injected intravenously cultures of streptococci into the animals, can have any bearing on the disease of rickets in children. In these experiments the animals were made very ill, developed high pyrexia, swollen joints full of fluid, which were very painful, and often made it impossible for the animals to walk for ten to fourteen days, and crippled them for longer periods. Quite apart from the effect of the severe illness, the complete incapability of movement, following the streptococcal injections, would tend to increase rickets unless the animals were well fed. The experimental conditions of J. Koch's work are wholly artificial, and although they might lead to interesting information concerning the effect of illness on calcification processes they do not prove the hypothesis as to the infective origin of rickets.

The experimental results of Morpurgo (35) are more difficult to explain. He obtained rachitic changes in a large proportion of rats as the result of injecting cultures of a diplococcus, previously isolated from laboratory rats among whom a spontaneous outbreak of late rickets had appeared. In these experiments there was a long latent period of 2 to 3 months before there was any evidence of abnormality in the young inoculated rats. Schmorl states that he has produced rickets in two only out of many experimental rats by inoculating them with tissues of rachitic animals. It is necessary to add that but little attention was given to dietetic conditions in any of these experiments on infection.

It seems to me probable that the support given by some clinicians to the infective hypothesis of rickets is due to their mixing up cause and effect. Instead of the catarrhal conditions, that so often develop

in rickety children, being the cause of rickets, both the susceptibility to catarrh and the rachitic condition are, to my mind, caused by wrong dieting such as has been described in this paper. It is impressive to observe the difference in susceptibility to diseases like distemper and mange in puppies which are receiving rickets-producing and those eating good diets. The same difference in the resistance and rate of cure is seen when puppies on these diets develop an intercurrent infection. In fact it has become my practice to kill off all the puppies as soon as distemper is introduced into the kennels, because of the experience that the animals on the deficient diets will probably develop the disease severely.

SUMMARY.

All my experience is against the hypothesis that rickets is due to an infection, although it is probable that a superadded infective state interferes with calcification processes of bone, and makes the conditions more suitable for the development of the disease. The dietetic conditions suitable for the production of rachitic changes lowers the resistance of an animal very greatly to infective agencies of all kinds.

VIII. OTHER DEFECTS WHICH DEVELOP IN PUPPIES EATING RICKETS-PRODUCING DIETS

(a) Susceptibility to Anaesthetics.

The necessity of anaesthetizing animals for radiographic examination brought out a fact which is of practical importance and deserves further investigation. No systematic examination of the point has been made in this work, and I wish here only to record the fact that dietetic deficiencies introduce dangers into anaesthesia which do not appear to have been described.

For a few weeks after receiving the rickets-producing diets the puppies could be anaesthetized with fair safety, and reacted in a similar way to animals receiving fuller diets. They recovered from the anaesthesia in the ordinary way and proceeded to eat their diets. After the experimental conditions had continued from six to ten weeks, according to their severity, it became dangerous to anaesthetize these puppies. The danger was of two kinds :

(1) Unless great care was used, the animals sometimes died during anaesthesia of heart failure.

(2) A more common occurrence was that after anaesthesia the animals never again became normal under the conditions of the experiment. Either they died within a few days or, more frequently, went off their food and lost weight and were therefore killed. This latter state, which was a kind of malaise, often continued for weeks. If the object of the experiment had been attained, they could usually be brought back to health by changing the diet to a good one, and especially by giving them plenty of whole milk.

In anaesthetizing the animals it was customary to inject morphine (about $\frac{3}{4}$ c.c. of a 1 per cent. solution per kilogram body weight),

and to use a mixture of chloroform and ether (2 to 3). In specially dangerous cases ether alone was used. In later experiments preliminary morphine injections were often omitted, but this did not make much difference to the general results recorded above. Omission of the morphine naturally decreases the danger to respiratory failure, but this is not a great advantage, partly because it is easy to keep a careful look-out for this incident, but also because, if noticed early, recovery usually follows artificial respiration. It is heart failure which is the crux of the matter. It may come on at any time during anaesthesia; it is not easy to observe, and recovery after its onset is specially difficult to bring about. It seems as if the diets deficient in the anti-rachitic vitamine and rich in bread produce pathological changes in the heart, possibly of the same nature as those changes which develop in the voluntary muscle of rachitic animals, so that it becomes easily knocked out by poisonous substances like anaesthetics.

What the chain of events happens to be in the general illness of the rachitic animals after recovery from anaesthesia, I do not know at the present time, but few puppies which have developed severe rickets, ever recover to the state present before anaesthesia unless the diet is changed.

(b) Nervous Symptoms.

The appearance of nervous symptoms in puppies eating defective diets is common. They can be classified into three main groups:

(1) In animals confined to their kennels, a common occurrence is that on being allowed freedom they are incapable of running straight. Often they run round and round in a small circle. At other times they behave as if intoxicated, starting off for a few steps in one direction, swaying in another direction, and then falling over. This may be repeated again and again until the puppy comes to the conclusion that it cannot arrive at any point it aims at and then it sits down. In both these cases it appears as if there were something wrong in the vestibular nerves or cerebellum.

(2) Paralysis especially of the hind legs. This condition was especially common in the experiments where casein was added to the diets, and sometimes even when the diet contained Fat-soluble A. Nor did recovery take place simply on removal of the casein. One of the most rapid cases of cure followed the removal of acid caseinogen and addition of whey to the diet—in this experiment the animal was eating butter (324). The addition of the ash of separated milk powder also brought about recovery in another animal (325). An abundance of whole milk quickly changes an animal incapable of movement into an active puppy, if the bone deformities allow activity. At all events the paralytic condition disappears. I do not know what pathological changes accompany this paralytic state, i.e. whether they are muscular or nervous. The curative effect of whole milk, whey or milk ash and butter suggests that the defect arises from some abnormality of calcium metabolism.

(3) Tetany and Convulsions. The association of these conditions with rickets is well known, and occasionally they were seen in the rachitic animals. This did not happen as frequently as might be

expected. Here again the addition of casein to a diet deficient in the anti-rachitic vitamine seemed to have a special power for calling forth tetany and convulsions. The addition of egg yolk to the diet of one dog suffering in this way did not bring about recovery, although a cure of the rachitic condition was started.

(c) **Keratomalacia and Diminished Resistance to Infection.**

A great deal has been written in recent years as to the susceptibility of animals eating defective diets to keratomalacia, and it is now placed among the 'deficiency diseases'. Surprisingly few of my animals developed the condition, but it was seen at different times in puppies eating linseed oil, cotton-seed oil, lard, autoclaved and oxidized butter as fat elements, also in pregnant bitches eating diets deficient in the Fat-soluble vitamine. As has been often observed by other workers in rats, children, and dogs, it cleared up quickly, if caught at an early stage, on the addition of cod-liver oil to the diet. In other untreated cases it rapidly went on to ulceration, suppuration, and blindness.

One case, lard (Exp. 295), cleared up without change of diet. I was impressed by the relation of the type of bedding used in the kennels to the development of the disease. When sawdust was used, the eye disease was more common. With straw and shavings the condition was but rarely found. It is difficult to avoid the conclusion that the irritating action of the sawdust on the eyes was a predisposing factor.

The variation in resistance of the various dogs to distemper, broncho-pneumonia, and mange dependent on the type of diet has already been mentioned.

SUMMARY.

Diets defective in quality but abundant in quantity may be responsible for the following conditions arising in animals :

- (1) Heart failure during anaesthesia and difficulty in recovery so that after anaesthesia they refuse food and lose weight.
- (2) Several types of nervous defects including inco-ordinated movements, paralysis, convulsions, and tetany.
- (3) Keratomalacia.
- (4) Increased susceptibility and lowered resistance to distemper and other catarrhal conditions, broncho-pneumonia, and skin affections like mange.

IX. DISCUSSION OF RESULTS

In this work an attempt has been made to examine the influence exerted by the various factors of diet and environment on the production of rickets in puppies, the observations being principally directed to the alterations in the calcification processes of growing bone produced by dietetic and other changes. It has been shown that many of the food elements exert a potent influence on the operation of bone calcification or on growth, or on both, and, moreover, that there is great interplay among these substances. So close, in fact, is this interrelation among the dietetic elements that a condition which

appears of prime importance at one time may sink into relative insignificance at another time. Although numerous experiments have been carried out testing the effect of one variable at a time, so many combinations and permutations are possible that much remains to be done. Until all the variations have been tested there is a danger that important points have been missed and the relative importance of the different effects wrongly evaluated.

The following conditions tend to prevent rickets in puppies :

- (1) Plenty of calcium and phosphorus in the diet.
- (2) Something associated with certain fats probably identical with the Fat-soluble vitamine.
- (3) Meat.
- (4) The possibility of exercise.

On the other hand, conditions which inhibit calcification or increase growth relatively to calcification so that defectively calcified bone results are :

- (1) A deficiency of calcium and phosphorus in diet.
- (2) A deficiency of fat containing the anti-rachitic vitamine in diet.
- (3) Excess of bread, other cereals, and carbohydrates.
- (4) Absence of meat.
- (5) Excess of the protein moiety of caseinogen free from calcium.
- (6) Confinement.

Of these conditions, probably the most common cause of rickets in children is a combination of relatively deficient anti-rachitic vitamine and excessive bread. In late and adolescent rickets I think it probable that deficient calcium in the diet is also a causative agent and is possibly the most important defect. It is to be remembered that, wherever there is growth of bone with large defective calcification, many of the clinical indications of rickets will develop.

Because of the inter-dependence of all these dietetic factors it is impossible to say what is the absolute amount of each necessary to produce the optimum results. It is a question of balance, and the greater the number of substances having an anti-rachitic effect that are eaten the less important are the remaining factors for the production of perfect bones.

The most interesting of the actions is the calcification influence exerted by the anti-rachitic vitamine. This action can be emphasized or antagonized by other conditions. If the diet contains a sufficiency of calcium and phosphorus, the presence of meat and the possibility of exercise make a small amount of the anti-rachitic vitamine very effective. On the other hand, excess of bread, causing the animal to put on weight rapidly, combined with confinement or some special condition, such as altering the caseinogen-calcium balance, make the anti-rachitic vitamine less effective. The aiding of and detracting from effectiveness by other dietetic constituents applies not only to the anti-rachitic vitamine but to other elements of diet, so that the so-called law of the minimum is inadequate to explain the problems of nutrition. The minimum of each substance for growth and perfect health varies with the amounts and kinds of other food elements eaten.

Up to the present but little light has been thrown on the problem as to the way in which the anti-rachitic vitamine acts. I have given some evidence that its influence on calcification is of an indirect nature. It is possible that closer examination of the accumulated material will make it possible to get a better insight into the mode of action of this substance. Until this evidence is forthcoming and further experiments are made, it is useless to discuss the action of anti-rachitic vitamine and its possible relation to organs of internal secretion, although the function of controlling calcification has been ascribed at one time or another to all the endocrine glands.

One other element of the diet concerned in calcification I have not discussed in this paper, viz. the anti-scorbutic vitamine. The brittle bones found in scurvy are sufficient evidence of the part played by this vitamine in the calcification of bone. Its absence from the diet appears to be associated with the actual removal of calcium salts from calcified bone, and, when a diet is relatively deficient in both anti-rachitic and anti-scorbutic vitamine, very bad calcification results. I have left this side of the question alone in this publication because of a desire to consider the disease of rickets uncomplicated by the scorbutic condition, and, for the past three years of the research, the diets have contained a constant additional amount of anti-scorbutic vitamine. In the case of children the diseases of rickets and scurvy are no doubt often associated. It is desirable that experiments should be carried out on the anti-scorbutic vitamine and its relation to the other elements of the diet. From observations made during the course of this research I think it probable that the anti-scorbutic vitamine does not hold the position of independence in the diet usually assigned to it.

I am not greatly concerned at present with the multifarious hypotheses as to the aetiology of rickets, for we have first to get definite evidence of the facts of the case. To combine the facts into a simple general hypothesis, at this stage of the work at least, seems impossible except to state that some elements of the diet assist in the calcification of bone (anti-rachitic vitamine), and others inhibit it (protein moiety of caseinogen in milk as prepared by acid precipitation of milk), while some increase growth and allow calcification processes to lag behind (bread and cereals, carbohydrate). The greater the growth the more necessary is it to have in the diet, and absorbed from the alimentary canal, substances which aid in calcifying bone, e. g. calcium, phosphorus, and anti-rachitic vitamine. If these latter substances are relatively deficient or defective in their action rickets will result. In addition to the dietetic elements there is the effect of exercise to be considered. This aids the processes of calcification. But, if a child is correctly fed, the question of exercise appears to me to be negligible, partly because exercise is not so necessary when calcification processes are strongly stimulated by the dietetic elements and partly because an adequately fed child will get exercise in the form of small movements under any conditions. If children are improperly fed, from the point of view of this research, they will almost certainly react, as shown in the case of puppies, by not taking exercise.

Although most of the facts described above will be accepted by

clinicians as applying to children, some points, more particularly as regards the action and distribution of the anti-rachitic vitamine, remain untried. Tests are now being carried out in Vienna by the Medical Research Council, and it will be interesting to see whether the results obtained are in accordance with those described in this paper. For it is upon the child that the final tests must be made, and it lies with the clinician to appraise the results of the experiments on animals described in this work.

Much of this research has been carried out at the Household and Social Science Department, King's College for Women (Univ. of London), and in the earlier days the actual feeding experiments were performed there. I wish to express my indebtedness to Miss Maude Taylor of Blundellsands for supplying facilities for this work. The greater part of the feeding of the puppies was done at the Field Laboratory, Cambridge, through the courtesy of the University Field Laboratory Committee. The extensive scale of the research was only rendered possible by the opportunities afforded by working under these conditions, where a large number of animals could be kept in the country.

Miss Margaret Higginton has had sole charge of the actual feeding and her patience and fortitude have brought the work through many difficulties. Some of the earlier radiographs were taken by Mr. Winch, of St. Thomas's Hospital, London, and some by Dr. Scales, of Addenbrook's Hospital, Cambridge, to both of whom I wish to express my indebtedness. Most of the radiographs were taken by my wife in the Physiology Laboratory of Cambridge University, through the kindness of Professor Langley. My wife has also been responsible for the general direction of the histological work, much of which she has carried out herself; but her chief help has been in constructive criticism throughout the whole research.

I wish also to thank Mr. G. H. Payne for his excellent work in the histology and photographic, especially the microphotographic, portions of this research.

APPENDIX

Since this paper was written researches on rickets and calcification processes in bones by McCollum and his co-workers have been published (38). In their work rats have been the experimental animals used and McCollum has confirmed the effect of cod-liver oil in stimulating calcification processes after the production of rickets by defective diets. In a still later paper (39) the production of rickets in rats by a combined deficiency of Fat-Soluble vitamine and phosphorus is described by the same authors.

I have also had the privilege of seeing results obtained by Professor Korenchevsky, working on behalf of the Medical Research Council at the Lister Institute, in which he produces rachitic changes in the bones of rats by diets deficient in both Fat-Soluble vitamine and calcium salts. This unpublished investigation of Professor Korenchewsky seems to confirm and extend many of the facts dealing with vitamins and calcium described above.

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ILLUSTRATIONS

FIGURES 1-129



FIG. 1. Child with rickets.



FIG. 2. Retriever with rickets.

F



FIG. 3. Exp. 176. Radiograph after 9 weeks of diet. Linseed oil 10 c.c. and extra separated milk 175 c.c.



FIG. 4. Exp. 177. Radiograph after 17 weeks of diet. Linseed oil 10 c.c.



FIG. 5. Exp. 178. Radiograph after 9 weeks of diet. Cod-liver oil 5-7.5 c.c.



FIG. 6. Exp. 179. Radiograph after 14 weeks of diet. Cod-liver oil 10-15 c.c.



FIG. 7. Exp. 186. Radiograph after 7 weeks of diet. Linseed oil 10 c.c.



FIG. 8. Exp. 187. Radiograph after 7 weeks of diet. Cotton-seed oil 10 c.c.



FIG. 9. Exp. 189. Radiograph after 10 weeks of diet. Pea-nut (arachis) oil 10 c.c.



FIG. 10. Exp. 190. Radiograph after 10 weeks of diet. Cod-liver oil 10 c.c.

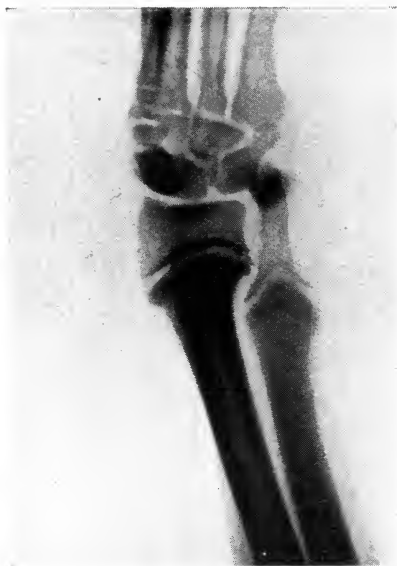


FIG. 11. Exp. 180. Radiograph after 18 weeks of diet. Suet 10 grm.



FIG. 12. Exp. 181. Radiograph after 14 weeks of diet. Lard 10 grm.



FIG. 13. Exp. 182. Radiograph after 14 weeks of diet. Butter 10 grm.



FIG. 14. Exp. 183. Radiograph after 14 weeks of diet. Babassu oil 10 grm.



FIG. 15. Exp. 220. Radiograph after 9 weeks of diet. Crushed palm-kernel 10 gm. (much bread eaten). Bad rickets.

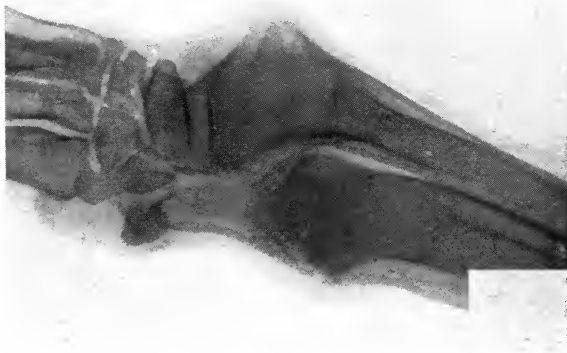


FIG. 16. Exp. 220. Radiograph 4 weeks after Fig. 15. Diet changed after Fig. 15 to 500 c.c. whole milk instead of 250 c.c. separated milk, palm-kernel oil stopped. Calcification at epiphyses renewed.



FIG. 17. Exp. 220. Radiograph 17 weeks after Fig. 16. Shafts of bones more bent in spite of healing processes.

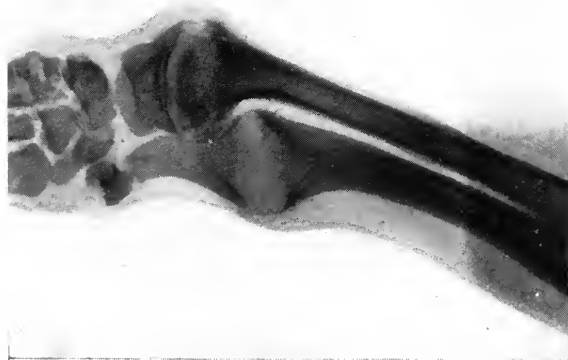


FIG. 18. Exp 223. Radiograph after 9 weeks of diet. Palm-kernel oil (extracted) 10 gm. Fairly bad rickets.

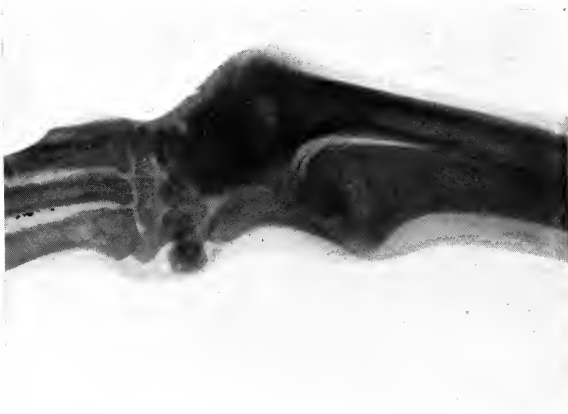


FIG. 19. Exp. 223. Radiograph 4 weeks after Fig. 18. No alteration of diet. Curative changes begun at epiphyses.



FIG. 20. Exp. 223. Radiograph 21 weeks after Fig. 19. Epiphyseal ends almost normal. Shafts bent and other evidences of abnormality of the bones.



FIG. 21. Exp. 221. Radiograph after 9 weeks of diet. Palm-kernel oil (crushed) 10 gm. Much less bread eaten than 220 (Fig. 15). Slight rickets.



FIG. 22. Exp. 221. Four weeks after Fig. 21. No alteration of diet. Curative changes at epiphyses.

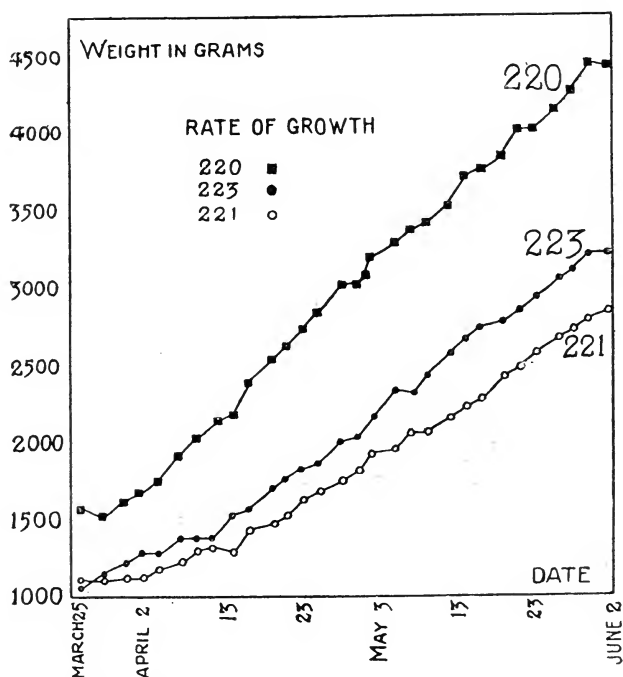
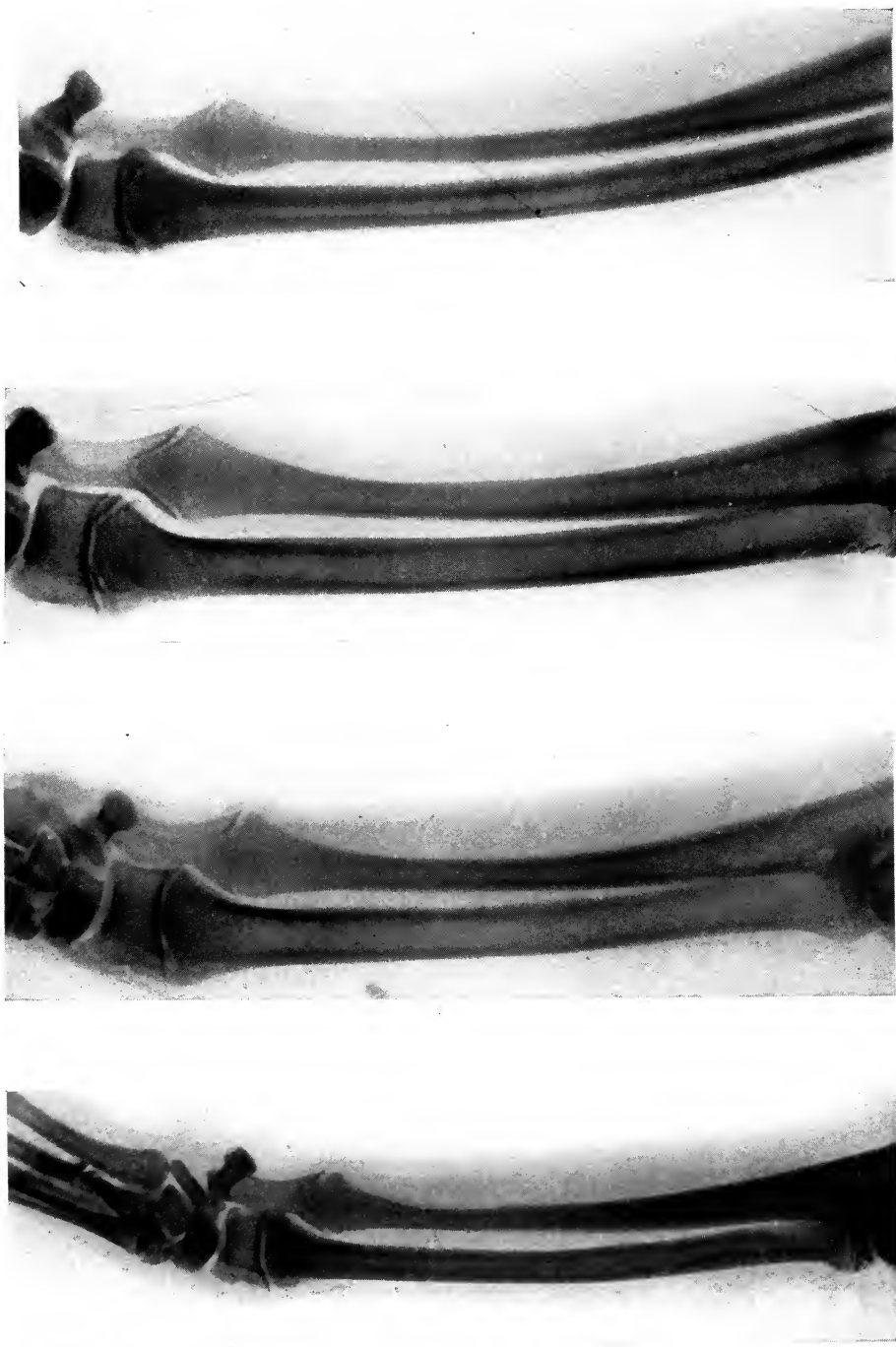


FIG. 23. Weight curves of Exps. 220, 223 and 221. The most rapidly growing puppy, 220 (most bread eaten), developed the worst rickets.



FIGS. 30, 31, 32, and 33. Expts. 264, 265, 266, and 267. Radiographs after 17 weeks of diet. 264 and 267, coco-nut oil 10 grm. 265 and 266, hydrogenated fat 10 grm. Puppies 3-4 months old when diet started. Not of same family. No signs rickets at epiphyses. Note relatively thicker periosteal bone of 264 and 267 (coco-nut) and smaller medullary cavity as compared with 265 and 266 (hydrogenated fat).



FIG. 34. Exp. 282. Radiograph after 8 weeks of diet. Lard 10 grm.



FIG. 35. Exp. 283. Radiograph after 8 weeks of diet. Suet 10 grm.



FIG. 36. Exp. 284. Radiograph after 8 weeks of diet. Bacon fat 10 grm.



FIG. 37. Exp. 285. Radiograph after 8 weeks of diet. Pea-nut oil 10 c.c.



FIG. 38. Exp. 328. Radiograph after 19 weeks of diet. Suet 10 gm.



FIG. 39. Exp. 329. Radiograph after 19 weeks of diet. Lard 10 gm.



FIG. 40. Exp. 303. Radiograph after 5 weeks of diet. Cod-liver oil 10 c.c.

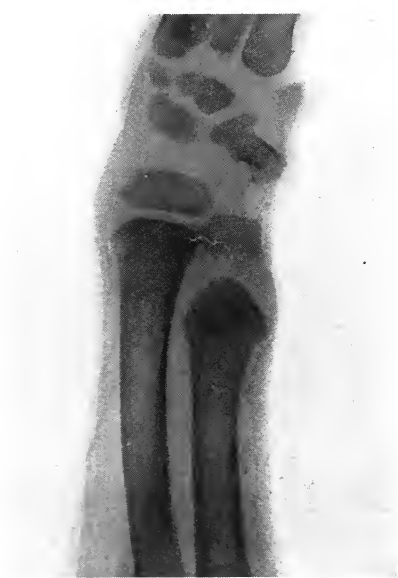


FIG. 41. Exp. 304. Radiograph after 5 weeks of diet. Rape-seed oil 10 c.c.

Of the radiographs, Figs. 40-45, olive oil (Fig. 43) shows the worst rickets, lard (Fig. 44) next. Cod-liver oil (Fig. 40) is normal and the others are intermediate.



FIG. 42. Exp. 305. Radiograph after 5 weeks of diet. Cotton-seed oil 10 c.c.



FIG. 43. Exp. 306. Radiograph after 5 weeks of diet. Olive oil 10 c.c.



FIG. 44. Exp. 307. Radiograph after 5 weeks of diet. Lard 10 gm.

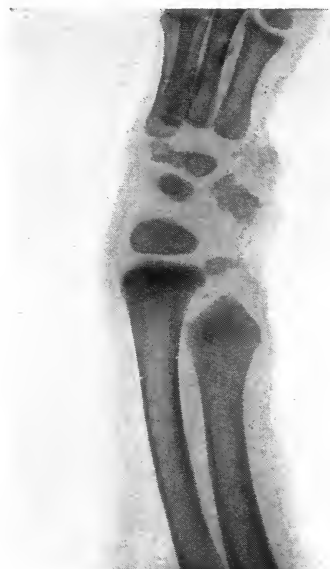


FIG. 45. Exp. 308. Radiograph after 5 weeks of diet. Bacon fat 10 gm.



FIG. 46. Exp. 321. Radiograph after 8 weeks of diet. Autoclaved and oxygenated butter (120°, 4 hours) 5-10 gm. No 'casein' (acid caseinogen).



FIG. 47. Exp. 322. Radiograph after 8 weeks of diet. Fresh butter 5-10 gm. Acid caseinogen 10-20 gm.



FIG. 48. Exp. 323. Radiograph after 8 weeks of diet. Autoclaved and oxygenated butter (120°, 4 hours) 5-10 gm. and acidic caseinogen (alcohol extracted) 10-20 gm.

Remarks on radiographs 46-50:

Comparison of heated and oxygenated butter with fresh butter, see Figs. 46 and 50. The heated butter has lost some anti-rachitic action.



FIG. 49. Exp. 324. Radiograph after 8 weeks of diet. Fresh butter 5-10 gm and acidic caseinogen (alcohol extracted) 10-20 gm.



FIG. 50. Exp. 325. Radiograph after 8 weeks of diet. Fresh butter 5-10 gm. No acidic caseinogen.

For action of acidic caseinogen, compare Fig. 48 with Fig. 46, also Figs. 47 and 49 with Fig. 50. The acidic caseinogen has greatly increased the rachitic condition.

For comparison of unextracted acidic caseinogen with alcohol extracted acidic caseinogen, see Figs. 47 and 49. There is but little difference between these radiographs both being bad from the point of view of rickets.



FIG. 51. Exp. 144. Radiograph taken some time after death. Length of experiment 5 months. Rapidly growing puppy, 50 gm. lean meat, 10 c.c. linseed oil. Much bread eaten.



FIG. 52. Exp. 336. Radiograph after 10 weeks of diet. Cod-liver oil 10 c.c.



FIG. 53. Exp. 337. Radiograph after 10 weeks of diet. Cod-liver oil (heated 120°C . 4 hours and oxidized) 10 c.c.



FIG. 54. Exp. 338. Radiograph after 10 weeks of diet. Pea-nut (arachis) oil 10 c.c.



FIG. 55. Exp. 339. Radiograph after 10 weeks of diet. Olive oil 10 c.c.



FIG. 56. Exp. 340. Radiograph after 10 weeks of diet. Coco-nut oil 10 gm.



FIG. 57. Exp. 341. Radiograph after 10 weeks of diet. Cotton-seed oil 10 c.c.

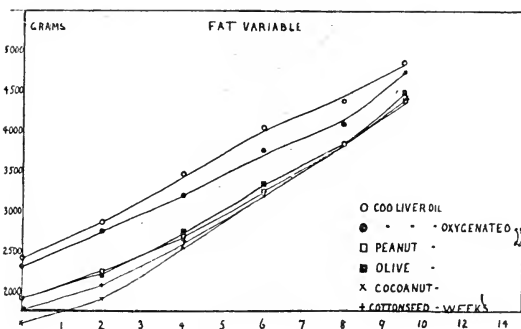


FIG. 58. Weight curves of 336-341. Note parallel rates of growth. All elements of diet quantitatively controlled. Type of fat only varying.

In Exps. 336-341 olive oil (339) had the worst rickets, coco-nut (340) and cotton-seed (341) slight rickets. A month after these radiographs were taken pea-nut (338) had developed some rachitic changes whereas cod-liver (336) and autoclaved cod-liver (337) remained normal throughout the experiment. All these animals were confined.



FIG. 59. Exp. 172. Radiograph after 13 weeks of diet. 10 gm. butter and 10 c.c. linseed oil. Butter has given protection. Compare with Figs. 60 and 61.



FIG. 60. Exp. 174. Radiograph after 13 weeks of diet. 10 gm. lean meat, 10 c.c. linseed oil. Rickets—but meat has had some anti-rachitic effect. See Fig. 7.



FIG. 61. Exp. 175. Radiograph after 13 weeks of diet. 50 gm. lean meat, 10 c.c. linseed oil. Rickets but slightly less than 174 (Fig. 60) which received 10 gm. meat.



FIG. 62. Exp. 351. Radiograph after 8 weeks of diet. 20-30 grm. meat, 10 c.c. linseed oil. Complete freedom (special muzzle) in open air during daytime; outside kennel at night.



FIG. 63. Exp. 352. Radiograph after 8 weeks of diet. No meat, 10 c.c. linseed oil. Complete freedom (special muzzle) in open air during daytime; outside kennel at night.



FIG. 64. Exp. 353. Radiograph after 8 weeks of diet. 20-30 grm. meat, 10 c.c. linseed oil. Confined, outside kennel.



FIG. 65. Exp. 354. Radiograph after 8 weeks of diet. No meat, 10 c.c. linseed oil. Confined, outside kennel.

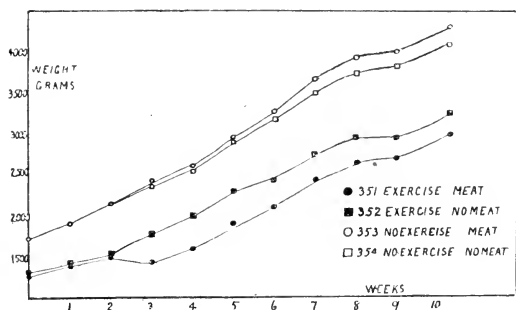


FIG. 66. Weight curves 351, 352, 353, and 354.



FIG. 67. Exp. 299. Radiograph after 13 weeks of diet. Meat protein 20 gm., linseed oil 10 c.c.



FIG. 68. Exp. 300. Radiograph after 13 weeks of diet. Cascin (edible, alkaline) 20 gm., linseed oil 10 c.c.



FIG. 69. Exp. 301. Radiograph after 13 weeks of diet. No extra protein, linseed oil 10 c.c. Control to 299 and 300.



FIG. 70. Exp. 299. Radiograph after 18 weeks of diet. Compare Fig. 67. Rickets slight and about the same.



FIG. 71. Exp. 300. Radiograph after 18 weeks of diet. Compare Fig. 68. Rickets slightly improved. Animal off diet.



FIG. 72. Exp. 301. Radiograph after 18 weeks of diet. Compare Fig. 69. Rickets progressed. Now distinctly worse than 299 (Fig. 70).



FIG. 73. Exp. 320. Radiograph after 15 weeks of diet. 75 gm. bread. Slight rickets.



FIG. 74. Exp. 319. Radiograph after 15 weeks of diet. 150-200 gm. bread. Fairly bad rickets.

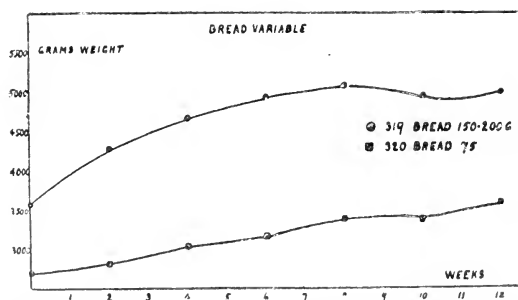


FIG. 75. Weight curves of 319 and 320. (Bread variable experiment.)



FIG. 76. Exp. 333. Radiograph after 15 weeks of diet. Linseed oil 10 c.c., 50 gm. bread. Slight rickets.



FIG. 77. Exp. 334. Radiograph after 15 weeks of diet. Linseed oil 10 c.c., 90-100 gm. bread. Worse rickets than 333 (Fig. 76).



FIG. 78. Exp. 335. Radiograph after 15 weeks of diet. Linseed oil 10 c.c., 150-180 gm. bread. Bad rickets.

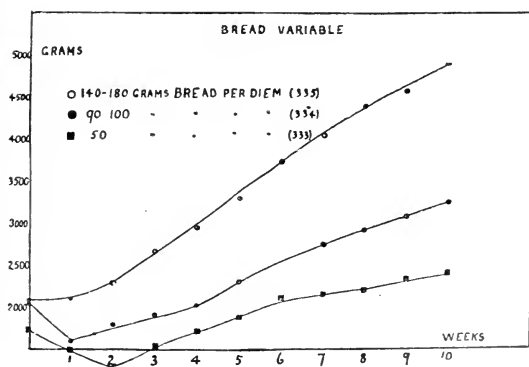


FIG. 79. Weight curves of 333 (Fig. 76), 334 (Fig. 77), and 335 (Fig. 78). (Bread variable experiment.)



FIG. 80. Exp. 322. Radiograph after 2 weeks of altered diet—13 weeks after beginning of experiment. Bread greatly reduced, butter increased to 15 gm. For previous diet see notes Fig. 47.



FIG. 81. Exp. 322. Radiograph 3 weeks after a further alteration in diet. Bread increased to 190 gm., acidic caseinogen replaced by 45 gm. separated milk powder. For previous diets see notes Figs. 80 and 47.



FIG. 82. Exp. 322. Radiograph 5 weeks after Fig. 81. No change of diet. No exercise. Evidence of further improvement in calcification and renewed growth of bone.



FIG. 83. Exp. 324 Radiograph after 2 weeks of altered diet—13 weeks after beginning of experiment. Bread increased to 190 grm., butter to 15 grm., and acidic caseinogen removed. For previous diet see notes Fig. 49.



FIG. 84. Exp. 324. Radiograph 4 weeks after Fig. 83. Diet unaltered. Healing processes brought about by removal of acidic caseinogen evident.



FIG. 85. Exp. 324. Radiograph 5 weeks after further alteration in diet. Whey from 450 c.c. separated milk added. For previous diets see notes of Figs. 83 and 49. Healing process quite advanced. Before addition of whey this animal was paralysed in hind limbs. No exercise.



FIG. 86. Exp. 325. Radiograph 16 days after altered diet. Thirteen weeks after beginning of experiment. Diet now contains 15 grm. acidic caseinogen, 15 grm. butter, 170 grm. bread. For previous diet see notes of Fig. 50. Rickets has now developed, compare with Fig. 50.



FIG. 87. Exp. 325. Radiograph 4 weeks after Fig. 86. Rickets has further progressed.



FIG. 88. Exp. 325. Radiograph after 5 weeks of a further alteration in diet. Acidic caseinogen removed, bread raised to 190 grm. and the ash of 45 grm. of separated milk powder added. For previous diets see notes of Figs. 86 and 50. Recovery process well started by change of diet. There was also improvement in paretic condition. No exercise.



FIG. 89. Exp. 198. Radiograph after 3 months of a good diet and confinement. Whole milk 250 c.c., meat 20 grm., cod-liver oil 5 c.c. Normal.

FIG. 90. Exp. 198. Photograph of puppy after confinement on a good diet (see notes Fig. 89).



FIG. 91. Exp. 198. Radiograph 15 months after change to defective diet—separated milk, linseed oil, &c. Bones have remained normal. For previous diet see notes of Fig. 89. Outside kennel. Compare with bones of 192 (Fig. 99). Periosteal bone is thick and the marrow cavity small as compared with 192 (Fig. 99).



FIG. 92. Exp. 199. (Brother of 198.) Radiograph after 10 weeks of diet. Linseed oil, &c. Complete freedom with special muzzle during daytime.



FIG. 93. Exp. 199. Radiograph 4 weeks after Fig. 92. No alterations in diet or environment. Recovery processes started at epiphyses.



FIG. 94. Exp. 199. Radiograph 4 weeks after Fig. 93. Conditions unaltered. Further recovery at epiphyses (self cure). In spite of recovery of calcification at epiphyses the calcium of the periosteal bone remained low.



FIG. 95. Exp. 193. Radiograph after 17 weeks of diet. Linseed oil, &c. Confined. Bad rickets.



FIG. 96. Exp. 192. (Brother of 193.) Radiograph after 17 weeks of diet. Linseed oil, &c. Exercise. Bad rickets but not quite as bad as 193.

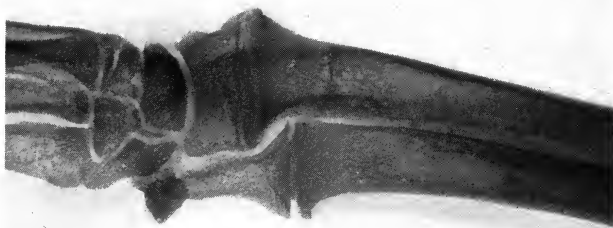


FIG. 97. Exp. 192. Radiograph 7 weeks after alteration to good diet. Cod-liver oil and meat instead of linseed oil. On deficient diet puppy became incapable of moving about but made rapid recovery on change of diet.



FIG. 98. Exp. 192. Radiograph 4½ months after Fig. 97. Diet unaltered. Further recovery at epiphyses but shafts of bones bent.

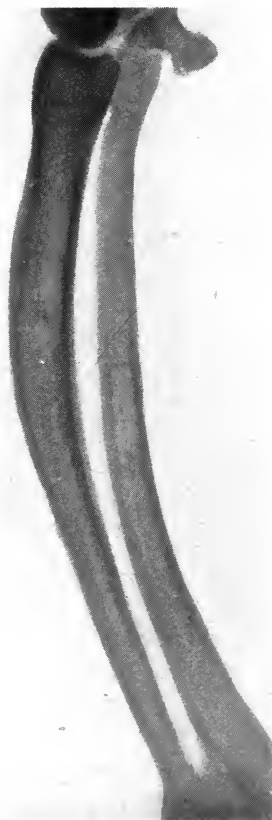


FIG. 99. Exp. 192. Radiograph 6 months after alteration back to defective diet. Linseed oil in place of cod-liver oil. For previous diets see notes, Figs. 97 and 96. No evidence of further rickets. Note thin periosteal bone and thick marrow cavity as compared with Exp. 198, Fig. 91.



FIG. 100. Exp. 250. Radiograph after 9 weeks of diet. Linseed oil 10 c.c., casein 20 grm. (edible, alkaline). Bad rickets.



FIG. 101. Exp. 250. Radiograph 4 weeks after change of diet. Casein removed and 2 egg yolks added. Calcification at epiphyses renewed. Compare with Figs. 103 and 105. No exercise since change of diet.



FIG. 102. Exp. 251. Radiograph after 9 weeks of diet. Linseed oil 10 c.c. No exercise. Bad rickets.



FIG. 103. Exp. 251. Radiograph 4 weeks after change of diet. Two whites of eggs added. Rachitic condition stationary. Compare with Figs. 101 and 105. No exercise.

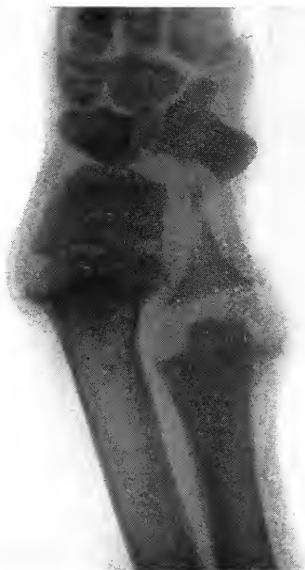


FIG. 104. Exp. 252. Radiograph after 9 weeks of diet. Linseed oil 10 c.c. Exercise. Rickets but not as advanced as Exp. 251 (Fig. 102).



FIG. 105. Exp. 252. Radiograph 4 weeks after Fig. 104. No change in diet. Rickets more pronounced. No exercise last 4 weeks. Control to 250 and 251.



FIG. 106. Exp. 205. Radiograph after 16 weeks of diet. Linseed oil, &c. Rickets.



FIG. 107. Exp. 205. Radiograph 26 days after Fig. 106. 15 c.c. cod-liver oil substituted for linseed oil. Curative changes have commenced at epiphyses.



FIG. 108. Exp. 213. Radiograph after 13 weeks of diet. Rape-seed oil, &c. Exercise. Rickets.



FIG. 109. Exp. 213. Radiograph $3\frac{1}{2}$ weeks after change of diet, 20 gm. butter. No exercise. Curative changes are obvious.



FIG. 110. Exp. 342. Radiograph after 12 weeks of diet. Olive oil, &c. and 5-20 grains thyroideum siccum.



FIG. 111. Exp. 343. Radiograph after 12 weeks of diet. Olive oil, &c., no thyroideum siccum.

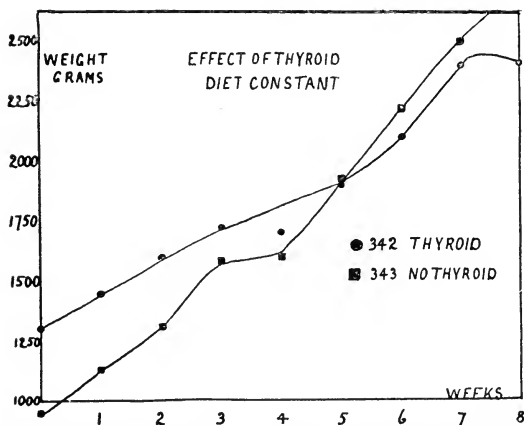


FIG. 112. Weight curves of Exp. 342 and 343 (Figs. 110 and 111). Diets eaten of same energy value. Weight put on by 342 (thyroid) more slowly than 343 (control).



FIG. 113. Exp. 350. Radiograph after 14 weeks of diet and confinement. 'Good' diet. Whole milk 200 c.c., meat 20 gm., dog biscuit 50-150 gm. Quite normal in spite of confinement.



FIG. 114. Microphotograph $\times 17$. Showing epiphyseal cartilage at lower end of ulna of a puppy on rickets-producing diet. (Linseed, &c.)

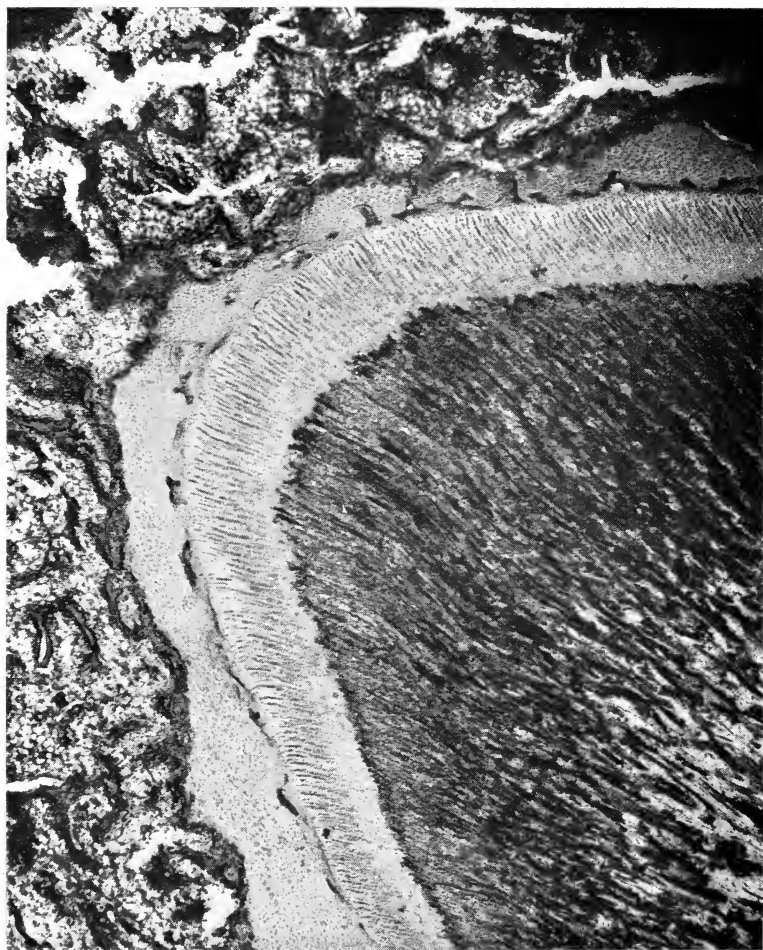


FIG. 115. As 114, but normal owing to 'good' diet. (Suet, &c.)

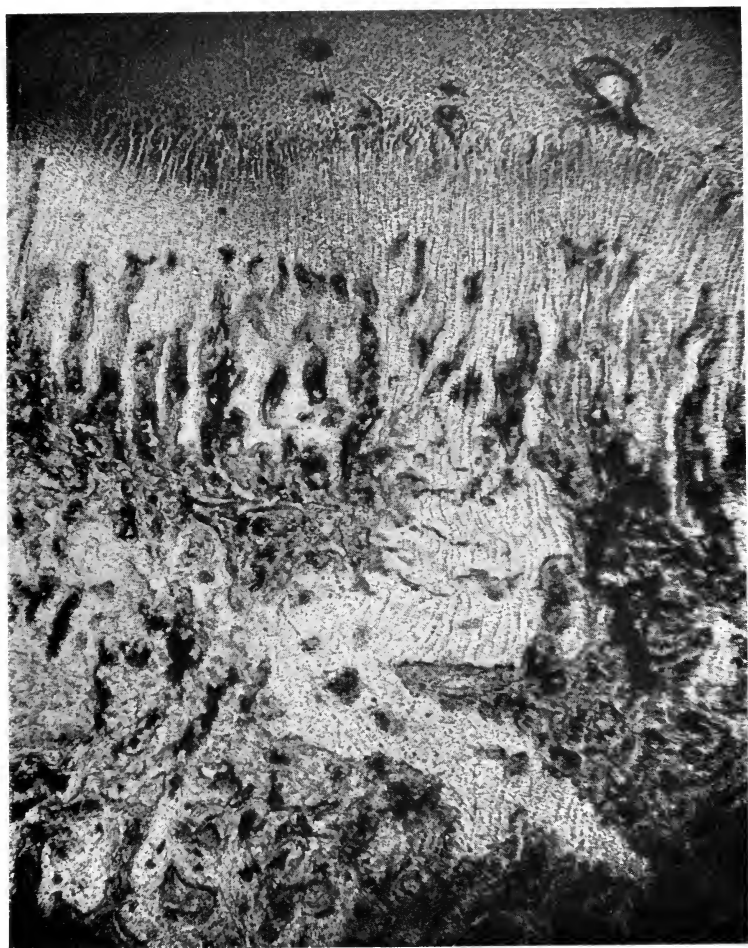


FIG. 116. Microphotograph $\times 28$. Costochondral junction of rickety puppy (linseed, &c.). Note hypertrophy of proliferating cartilage and irregular invasion of marrow vessels.

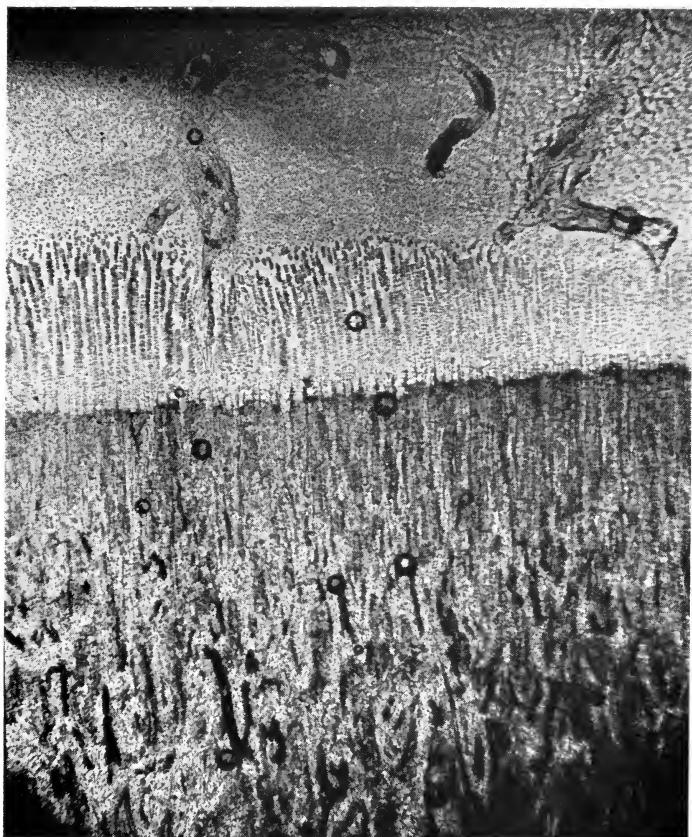


FIG. 117. As 116, but of normal puppy on 'good' diet (cod-liver oil, &c.).

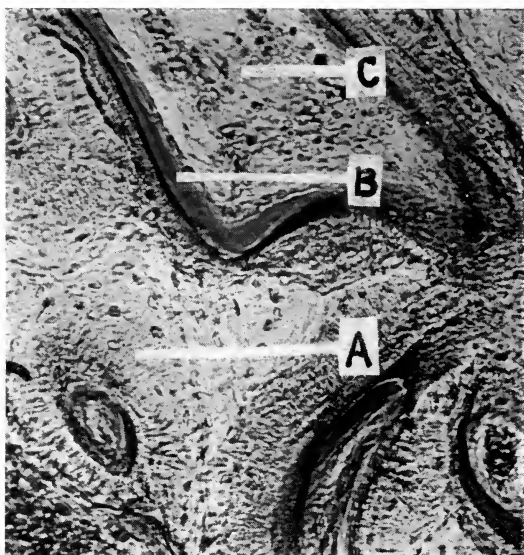


FIG. 118. Microphotograph $\times 200$. Ground section of femur shaft in the region of attachment of muscle. (Undecalcified—Weil's method.) Showing bone (A), osteoid tissue (B), and marrow (C). Rickets-producing diet, but small amount of bread (Exp. 320).

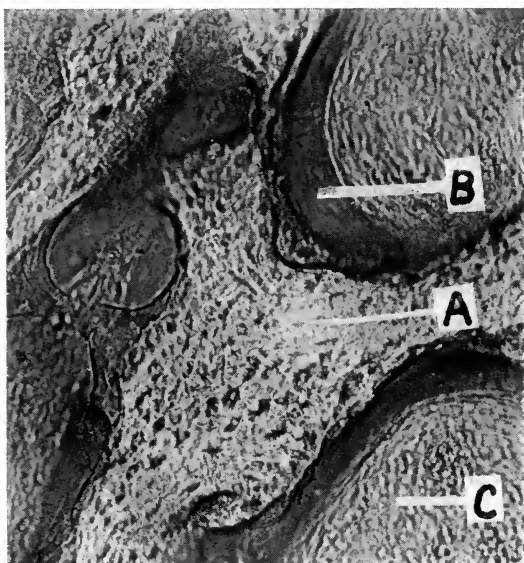


FIG. 119. As 118, but large amount of bread eaten by puppy (Exp. 319). Abundant osteoid tissue (B) evident.

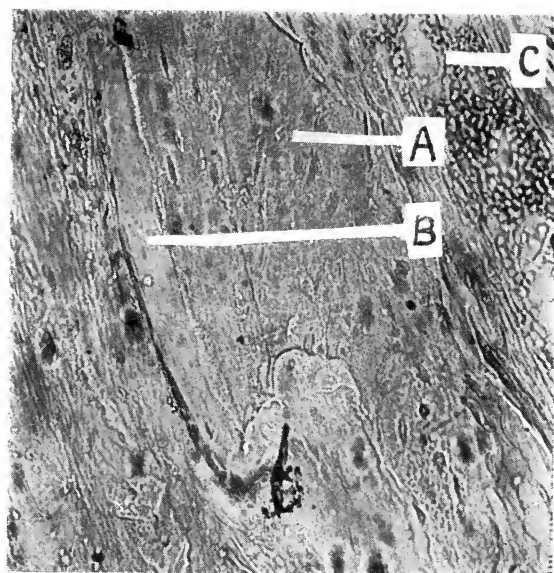


FIG. 120. Microphotograph $\times 200$. Rib partially decalcified by Müller's solution and stained with methylene blue. Rickets-producing diet Bone (A), osteoid tissue (B), marrow (C).

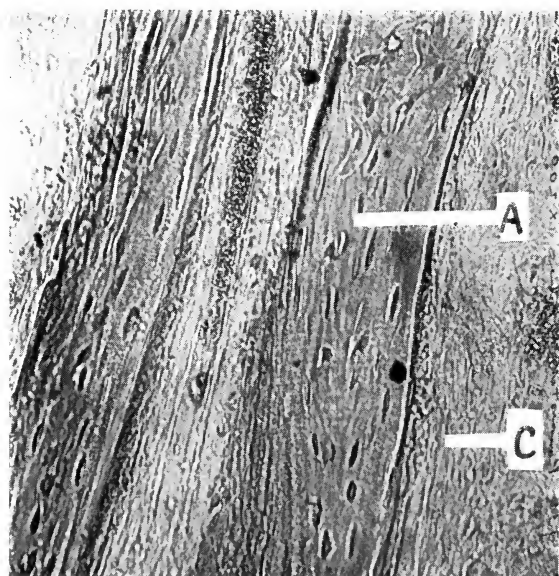


FIG. 121. As 120, but normal bone. Bone (A), marrow (C).

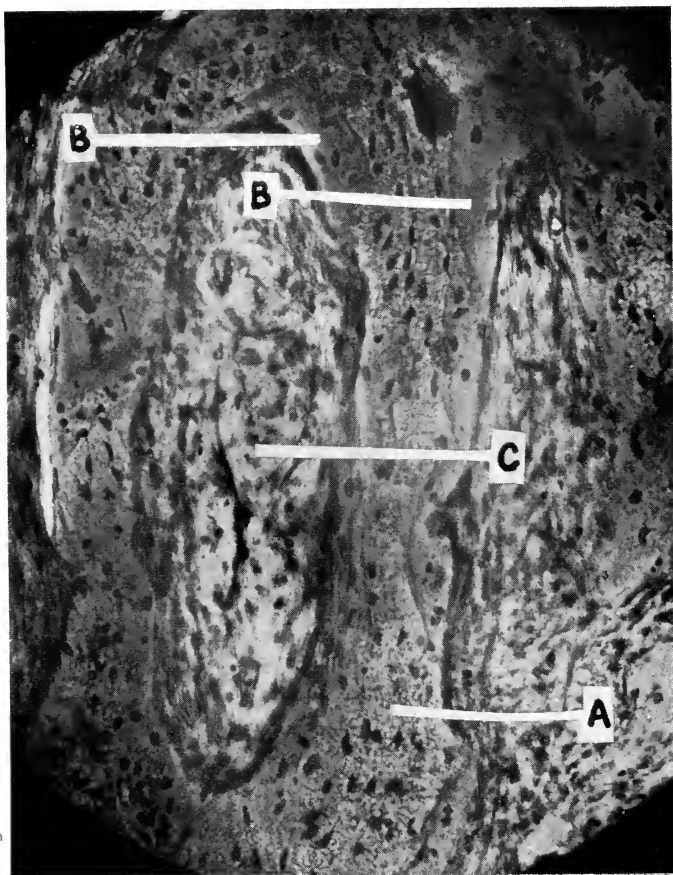


FIG. 122. Microphotograph $\times 200$ of decalcified section of rachitic rib stained by Schmorl's thionin method. Bone (A), osteoid tissue (B), and marrow (C). This method brings out clearly the cells both in the bone and osteoid tissue. Note the comparatively small number of cells with few canaliculi in the osteoid tissue.

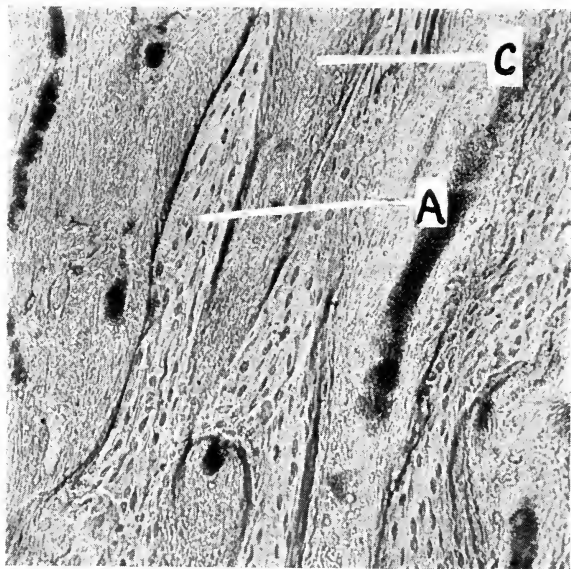


FIG. 123. Microphotograph $\times 100$ of partially decalcified section of normal rib. Pommer's ammonia carmine method. Shows bone (A) and marrow (C).

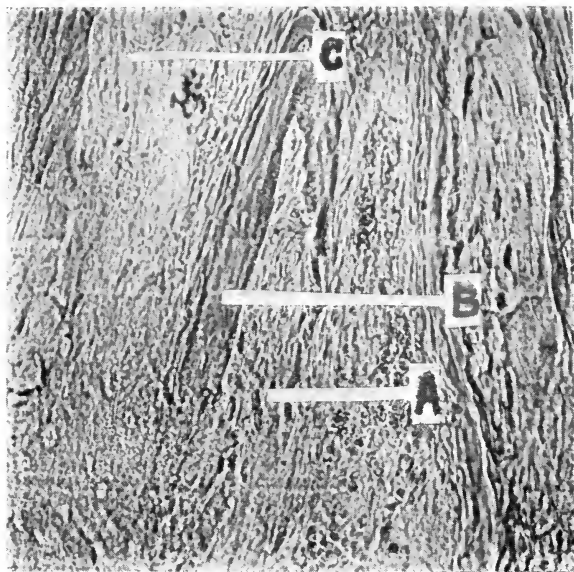


FIG. 124. As 123, but 200 magnification. Rachitic rib. Shows bone (A), osteoid tissue (B) and marrow (C). Fibrillar nature of osteoid tissue brought out.

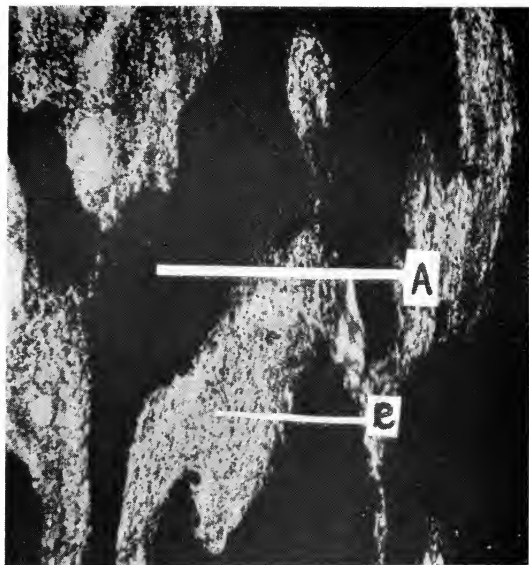


FIG. 125. Good diet (Exp. 336). No osteoid tissue

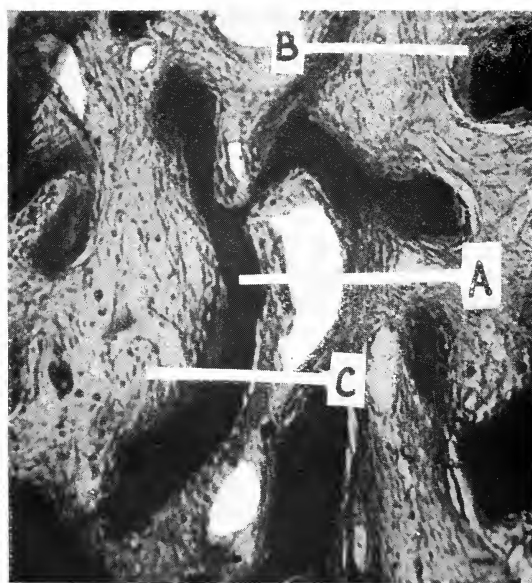


FIG. 126. Linseed oil and little bread (Exp. 333). Some osteoid tissue.
Note, magnification slightly higher than Figs. 125 and 127.

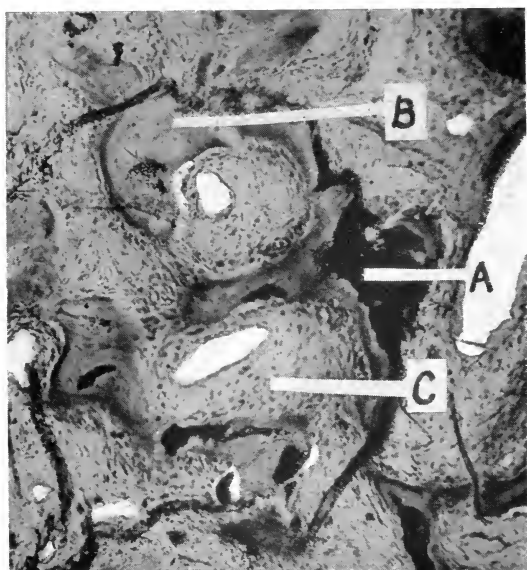


FIG. 127. Exp. 335. Linseed oil and large amount of bread. Much osteoid tissue.

FIGS. 125, 126, and 127. Microphotographs of sections of ribs of about the same thickness and from corresponding regions. Sections (undecalcified) cut in gum and stained with silver nitrate and eosin. Calcified bone black. Bone (A), osteoid tissue (B), and marrow (C).



FIG. 128.

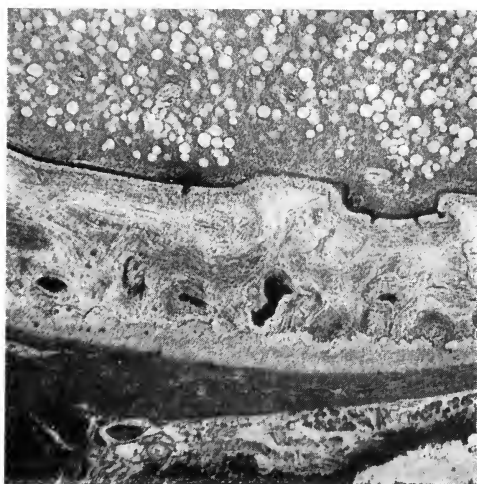


FIG. 129.

FIGS. 128 and 129 Microphotographs of ground sections (undecalcified) of corresponding parts of the femur shafts of Exp. 303, Fig. 128 (cod-liver oil), and 304, Fig. 129 (rape-seed oil). Periosteal bone thick and well formed in 303, but thin and poorly formed in 304.

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